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Nutrient and organic carbon input and abundance in supraglacial environments

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Abstract

Supraglacial environments are vital to glacial and ice sheet systems as they collect atmospheric and windblown inputs, are the locus of meltwater production and are a key nutrient source to englacial, subglacial and downstream environments. They have also been found to host abundant and diverse microbial communities focused in three main habitats; snow packs, bare ice and cryoconite holes. Yet due to the harsh conditions of this environment, microbial activity was considered of little global importance, particularly to biogeochemical cycles. Recently, large scale blooms of supraglacial microbial communities, in areas such as the Greenland Ice Sheet Dark Zone, an annually reoccurring area of decreasing surface albedo due to large scale algal blooms along the west-coast, have begun to change this assumption. In an effort to enhance the current understanding of nutrient cycling in supraglacial environments and the influence of resident microbial communities, comprehensive datasets of snow, ice and meltwater from multiple ablation seasons were collected from the Greenland Ice Sheet Dark Zone. A snow incubation experiment was also conducted to investigate microbial nutrient cycling under simulated polar winter conditions. The data indicate microbially mediated nutrient cycling in supraglacial environments during both the polar winter and main growth season, having implications for nutrients released from the snow pack during spring thaw and nutrients later exported from the supraglacial environment. Results from the spring thaw on the Greenland Ice Sheet (GrIS) show the snow pack to be an important source of nitrogen to the supraglacial environment, which may act as a stimulant for the annual algal bloom initiation. After the development of the algal bloom, during the peak of the growth season, the data reveal bare ice surfaces to be areas of high dissolved organic matter accumulation as glacier algae cycle nutrients from the inorganic to the organic phase more efficiently then heterotrophs remineralize the organic matter. Glacier algae blooms also depict a potential retention ability for maintaining dissolved organic nutrients at the surface. As a result, nutrient export to downstream environments is likely to be pulsed rather than constant, as well as rich in labile, dissolved organic nutrients, potentially impacting chemoheterotroph and chemoorganotroph community activity in subglacial environments. Further, data from early ice melt during spring thaw on the GrIS indicate dissolved organic nutrients retained in the ice surface over winter from the previous glacier algal bloom, provide a further nutrient source for the following season algal bloom. As the GrIS is the largest contributor to cryospheric sea level rise, and glacier algae blooms have been shown to significantly increase melt rates on the GrIS, it is imperative to better understand how they utilize and cycle nutrients in these surface ice habitats, as their expansion has critical implications for global sea level rise.

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Authors Declaration

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's *Regulations and Code of Practice for Research Degree Programmes* and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

Signed _____ Date _____

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Chapter 1: Introduction and Scientific Background

1.1 Introduction

At present, ice sheets and glaciers cover roughly 10 % of Earth's surface (Knight, 1999; Anesio and Laybourn-Parry, 2012) and play a significant role in shaping the physical landscape known today. It has been known for many years that glaciers and ice sheets are responsible for shaping many mountain ranges, affecting earth's albedo (Broccoli and Manabe, 1987) and contributing to global sea level change (Clark and Mix, 2002), yet, these large masses of ice were often thought of as desolate, static aspects of the Earth's surface. It is now known that ice sheets and glaciers are extremely dynamic ecosystems capable of sustaining unique microbial habitats (Hodson et al., 2008). In fact, areas of the Earth in which water is found in its solid, frozen form is now considered one of the Earth's five spheres, known as the cryosphere.

The cryosphere is shrinking in the current warming climate, with instrumental records from 1970 – 2010 showing an average global surface air temperature increase of ~ 0.6 °C, but with trends over the Arctic reporting temperature increases 3 – 4 times larger (Hansen et al., 2010), leading to sea ice mass loss across the Arctic on scales of 13.2 % per decade (Church et al., 2013). Even more impactful than sea ice loss is the melting of the ice sheets as they have far greater implications for Earth's physical landscape. Ice sheets sit atop land masses, causing their melt rates to have great influences over the rate of sea level change. For example, the Greenland Ice Sheet (GrIS) is the greatest contributor to global mean sea level rise, with reported increases from 0.09 mm yr^{-1} during 1991 – 2001 to 0.59 mm yr^{-1} for 2002 – 2011, and an average 7 m sea level rise were the GrIS to melt fully due to elevated temperatures and self-amplifying feedbacks (Church et al., 2013; Box and Sharp, 2017; Bamber et al., 2018).

A less obvious implication of a warming Arctic and increased melt rates is a change in nutrient cycling within the unique microbial habitats that reside in the cryosphere and downstream environments they feed. Research has recently increased surrounding biogeochemical processes occurring in subglacial (rock-glacier interface) environments beneath ice sheets and glaciers (Wadham et al., 2004; Hodson et al., 2008; Hodson et al., 2010a; Hood et al., 2015) as they have been found to source large amounts of nutrients and carbon to downstream and surrounding ocean ecosystems (Hodson et al., 2005; Lawson et al., 2014a; Lawson et al., 2014b; Musilova et al., 2017). While research of subglacial processes has increased, studies pertaining to supraglacial (the surface of glaciers and ice sheets) habitats remains lacking. Glacier and ice sheet surfaces serve a great importance for the rest of the glacial ecosystem as they are areas with direct interaction with the

atmosphere, collect air driven particles, host algal blooms, and are the locus of meltwater production that is subsequently delivered to englacial (within glacier ice) and subglacial systems. (Stibal et al., 2008b). Most recent research examining the microbiology and nutrient cycling within supraglacial environments has focused on habitats known as cryoconite holes, and the microbial life therein (Anesio et al., 2010; Anesio and Laybourn-Parry, 2012; Telling et al., 2012a). While these habitats are an important component of supraglacial environments, they typically only cover a small portion, 0.1-10 %, of glacier surfaces (Anesio et al., 2009). The remaining supraglacial environment is comprised of snow and bare ice, which both host diverse and dynamic communities (Yallop et al., 2012; Lutz et al., 2014; Lutz et al., 2016; Williamson et al., 2018). As glaciers and ice sheets continue to melt globally, their impact on downstream environments will increase significantly (Hodson et al., 2008; Tedstone et al., 2017). The more we understand how the microbial communities residing in these ecosystems influence factors such as carbon and nutrient cycling, the better we can predict their impact on downstream environments.

The following scientific background aims to provide an overview of the existing literature and highlight knowledge gaps surrounding supraglacial environments, with a focus on nutrient and carbon input and abundance as well as microbially mediated nutrient and carbon cycling. This chapter concludes by outlying the aims and objectives of this thesis and how it will fill the present gaps in the literature and further the current knowledge.

1.2 Scientific Background

1.2.1 Glaciers and ice sheets as part of the Cryosphere

1.2.1.1 Glaciers as a diverse ecosystem

Blooms of different coloured snow and glacier algae have been reported as far back as the writings of the ancient Greek Aristotle (Gentz-Werner, 2007; Lutz et al., 2014), yet because glaciers and ice sheets are commonly regarded as harsh environments, due to low nutrient concentrations, extreme variations in UV-radiation, low temperatures and low liquid water availability (Maccario et al., 2015; Ganey et al., 2017), microbial activity, abundance and diversity in these environments was not considered of global importance. Research over the past two decades, however, has begun to highlight the potential importance and impacts of supraglacial microbial communities on global nutrient cycles and the physical characteristics of the system they inhabit (Yallop et al., 2012; Takeuchi, 2013; Maccario et al., 2015; Takeuchi et al., 2015; Cook et al., 2017a; Stibal et al., 2017a; Tedstone et al., 2017; Ryan et al., 2018; Williamson et al., 2018; Williamson et al., 2019). It is now understood that supraglacial, subglacial and even englacial environments are capable of supporting

heterotrophic, autotrophic, fungal and viral communities (Fig. 1.1) (Hodson et al., 2008; Margesin et al., 2008; Maccario et al., 2015). Further, within the polycrystalline ice itself, ice veins, ~ 0.3 – 0.8 µm in size, which form in the pore space between ice crystals, have also been found to host abundant, 10^6 – 10^8 cells mL^{-1}, bacterial communities capable of living in an extremely solute rich and low pH environment (Mader et al., 2006; Barletta et al., 2012; Dani et al., 2012).

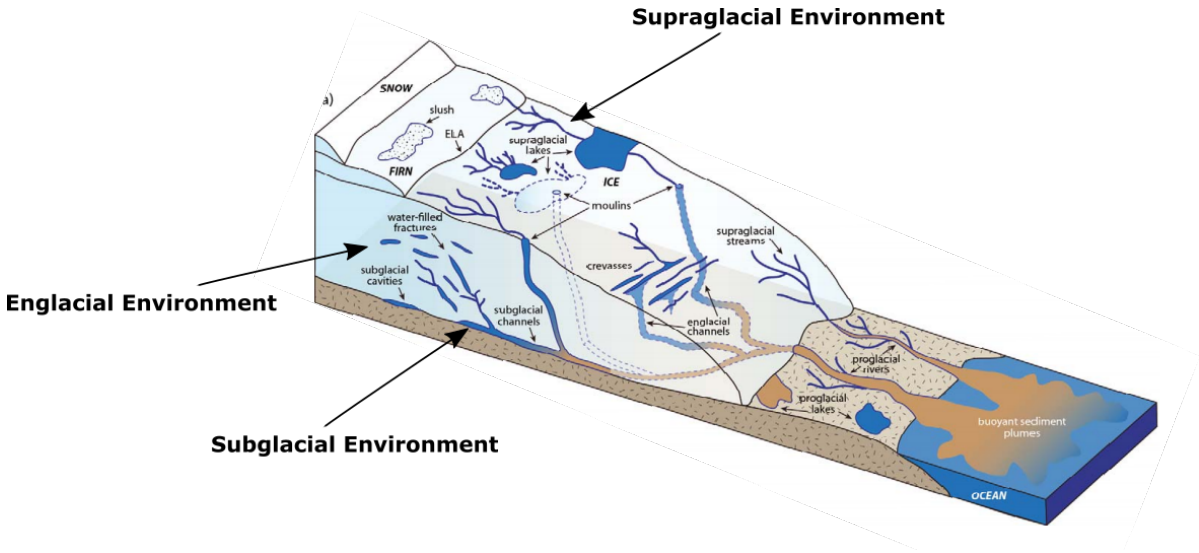

Figure 1.1: Schematic depicting the three main glacial ice environments (modified from Chu, 2014).

Many microorganisms living in these environments are psychrophiles, i.e. cold-adapted organisms with optimal growth temperatures below 15 ºC, while others are psychrotolerant, being capable of survival below 0 ºC, but with optimum temperatures for growth between 20 and 25 ºC (Morita, 1975). To cope with the extreme conditions characteristic of glacier and ice sheet environments, microorganisms may employ several adaptations including anti-freeze protein production, regulation of fatty acid production and increased mycosporine-like amino acid and carotenoid pigment production (Ryan et al., 2002; Feller and Gerday, 2003; Gorton and Vogelmann, 2003; Mundy et al., 2011; Remias et al., 2013; De Maayer et al., 2014). These vital adaptations allow psychrophiles and psychrotolerants to increase membrane fluidity or catalyse necessary chemical reactions within the cell for example, which would otherwise freeze or become inhibited by cold temperatures (Metz et al., 2001; Feller and Gerday, 2003). The production of carotenoid pigments, such as astaxanthin, and mycosporine-like amino acids provides a UV protectant, or self-shading mechanism, fundamental to the survival of supraglacial psychrophiles and psychrotolerants, which are subject to 24-hour intense UV radiation during polar summers (Ryan et al., 2002; Gorton and Vogelmann, 2003; Mundy et al., 2011).

Research involving microbial impact and interaction within different glacial environments has also increased. For example, studies of ice cores, which at present provide the greatest insight to englacial life, found bacteria to be present ($6.1 \times 10^7 – 9.1 \times 10^7$ cells ml^{-1} of melted ice) in ice cores collected from the GrIS at depths reaching 3042 m, though predominantly as "dwarf" cells (< 1 μm) (Sheridan et al., 2003; Miteva et al., 2004). The cells were also only reported to function at extremely low metabolic rates, sufficient for DNA and amino acid repair, with no evidence, to-date, of growth or reproduction (Tung et al., 2005). The main phyla detected included *Firmicutes*, *Actinobacteria*, *Bacteriodes* and *Proteobacteria* (Sheridan et al., 2003; Miteva and Brenchley, 2005), with significantly higher colony forming units detected in ice cores from the Guliya Ice Cap in Asia than ice collected from Antarctica, suggesting that a closer proximity to airborne particles from soil influences community composition (Anesio and Laybourn-Parry, 2012). Subglacial environments were also found to host microbial communities, though due to its low light and frequently low oxygen nature heterotrophic, chemoautotrophic prokaryotes and fungal species tend to dominate (Anesio and Laybourn-Parry, 2012). While current knowledge regarding the role of subglacial microbial communities on biogeochemical cycling is limited, some studies report that these communities aid in the dissolution of nutrients and solutes from the ice and even bedrock, leading to elevated nutrient export (Tranter et al., 2005; Tranter, 2006; Hodson et al., 2008), thus having importance for downstream fjord and ocean environments (Apollonio, 1973; Föllmi et al., 2009; Hodson et al., 2004; Wadham et al., 2013).

Supraglacial microbial communities tend to concentrate in three main habitats. First, is the snow pack, which has been found to host abundant life, in some cases as diverse as temperate soils and lakes (Larose et al., 2010). Second, and most heavily studied, are cryoconite holes, shallow water filled depressions in the ice surface containing organic rich sediments at their bottom. Finally, and at present the least understood, is the bare ice surface. Studies investigating these environments and their resident microbes are imperative, though still in their infancy as much remains unknown regarding how environmental factors and a current warming Arctic will influence their growth, and their impact on biogeochemical cycles. An overview of the current knowledge of these three main supraglacial habitats and their associated microbial communities are outlined below.

1.2.1.2 Cryoconite holes

Most early work on microbial life in supraglacial environments revolved around water filled holes in the ice surface known commonly as 'cryoconite holes', as they contained all required elements for life and they were ubiquitous across the cryosphere (Mueller et al., 2001; Christner et al., 2003; Tranter et al., 2004; Hodson et al., 2005; Anesio et al., 2009; Cook et al., 2016c). Typical cryoconite holes are < 1 m in diameter and < 0.5 m in depth (Anesio et al., 2009), but can vary greatly in size due to the

surrounding environment. Cryoconite holes are typically formed when dark sediment settles onto the ice surface, decreasing the surrounding albedo enough to melt into the ice surface (Takeuchi et al., 2001a).

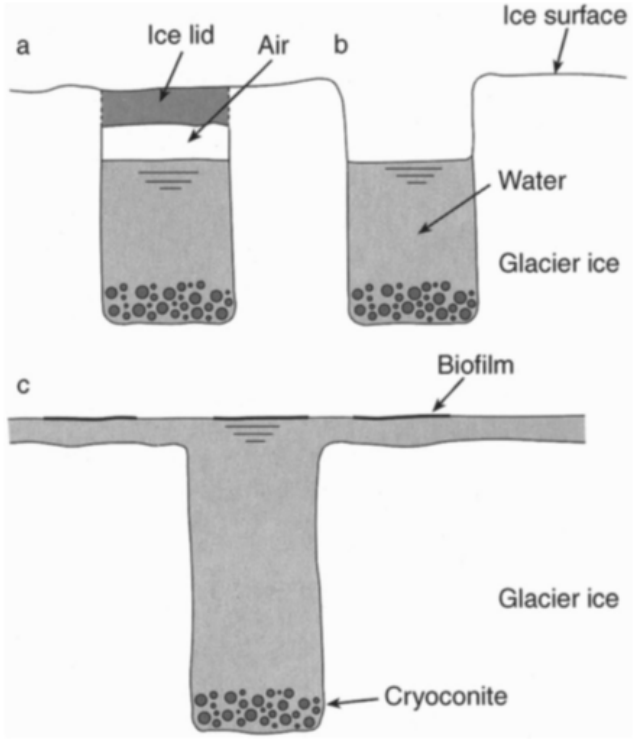

Figure 1.2: Different structures of a) closed, b) open and c) submerged cryoconite holes (Hodson et al., 2008).

Their formation is also greatly facilitated by microbes (Takeuchi et al., 2001a) such as bacteria, viruses and microalgae (Christner et al., 2003; Anesio et al., 2009; Bellas et al., 2013) that bind the sediment together through the production of glue-like extracellular polymeric substances (EPS) (Langford et al., 2010; Stibal et al., 2012a; Angelaalincy et al., 2017), producing an organic rich matrix, which melts collectively into the ice surface, resulting in a distinct habitat with active microbial cycling and biogeochemical processes (Säwström et al., 2002; Tranter et al., 2004; Hodson et al., 2005; Anesio et al., 2009). Studies of cryoconite holes across the cryosphere have concluded that of all the bacterial lineages present *Cyanobacteria, Proteobacteria* and *Bacteroidetes* are the most dominant (Christner et al., 2003; Edwards et al., 2011; Edwards et al., 2013) with Cyanobacteria genera such as *Phormidium* and *Nostoc* playing key roles as primary producers (Christner et al., 2003; Stibal et al., 2006). On Arctic and temperate glaciers and ice sheets, where higher temperature variation is common, cryoconite holes are typically open to the atmosphere and subject to melting out or flushing during periods of high melt (Fig. 1.2) (Mueller and Pollard, 2004; Hodson et al., 2008; Anesio et al., 2009; Hodson et al., 2010c; Telling et al., 2012b; Telling et al., 2014; Wadham et al., 2016). The melting and flushing of cryoconite holes commonly results in nutrients, dissolved organic matter and microbial transport to other glacial and downstream environments via water flow through

surface ice, supraglacial streams and moulin flow paths (Wynn et al., 2007; Cook et al., 2016a; Wadham et al., 2016). While nutrient concentrations in cryoconite holes are reportedly low (e.g. 48 – 132 μg l^{-1} dissolved inorganic nitrogen (DIN) and < 10 μg l^{-1} dissolved inorganic phosphorus (DIP) (Stibal et al., 2008b; Telling et al., 2011), when compared to other aquatic environments they are relatively enriched, particularly compared to other supraglacial habitats (Miteva, 2008; Stibal et al., 2008b; Telling et al., 2012b; Telling et al., 2014), consistent with the high rates of bacterial production reported for these habitats (e.g. NEP of cryoconite debris: 1.9 ± 0.88 – 22 ± 4.8 μg C g^{-1} day^{-1}) (Anesio et al., 2009; Stibal et al., 2012a). In fact, recent research has reported active carbon (Anesio et al., 2009; Telling et al., 2010; Telling et al., 2012a), nitrogen (Telling et al., 2011; Telling et al., 2012b) and phosphorus (Stibal et al., 2008b; Stibal et al., 2009) cycling within cryoconite holes, suggesting that the microbes residing in these habitats are well adapted for low nutrient concentrations.

While cryoconite holes cover only a small portion of glacier and ice sheet surfaces, (0.1 – 10 % of the total surface area: Anesio et al., 2009), biogeochemical cycling within these habitats may be significant at the regional and global scale. For example, one study up-scaled production rates measured within cryoconite holes for glaciers excluding Antarctica, and estimated the potential for a total carbon fixation of 64 Gg of carbon per year (Anesio et al., 2009), comparable to the productivity of other, more temperate, oligotrophic freshwater and soil environments (Stibal et al., 2012a). Nutrient cycling within cryoconite holes, which results in the storage of carbon, nitrogen and particulates (Anesio et al., 2009; Bagshaw et al., 2013; Bagshaw, 2018), has also been identified as a vital source of nutrients to nutrient-limited downstream environments, such as ice covered lakes in Antarctica, where nutrients derived from cryoconite holes stimulate primary production (Foreman et al., 2004; Bagshaw et al., 2013).

1.2.1.3 Snowpack

Permanent and seasonal snow packs cover 21 % of the Earth's surface, making them potentially key components in global biogeochemical cycles (Margesin et al., 2008; Maccario et al., 2015). Snow packs act as past climate and atmospheric proxies, surface albedo regulators and insulators for other Arctic environments. Although, unlike cryoconite habitats, the snow pack was largely overlooked as a potential hotspot for microbial activity, even though documentation of snow algal blooms have been reported as far back as ancient Greek Aristotle (Gentz-Werner, 2007). Yet, recent research into microbial abundance and taxonomy has revealed active snow pack microbial communities consisting of algae, bacteria and fungi (Carpenter et al., 2000; Larose et al., 2010; Harding et al., 2011; Takeuchi, 2013), with some snow packs demonstrated to be as diverse as temperate soils and lakes (Larose et al., 2010). Snow algae dominate most of the literature though, as they are the principle autotrophic life in

these environments (Hoham and Duval, 2001; Takeuchi, 2001b; Williams et al., 2003; Larose et al., 2010; Lutz et al., 2015; Lutz et al., 2016). These snow algae, now documented in snow packs worldwide (Hoham and Remias, 2020), are derived primarily from the family *Chlamydomonadaceae*, with green pigmented algae commonly dominated by *Microglena sp.* and red pigmented algae comprised predominantly of *Chlamydomonas nivalis* and *Chloromonas nivalis* (Williams et al., 2003; Takeuchi, 2013; Lutz et al., 2015). Cyanobacteria have also been found to comprise a portion of the autotrophic community in snow packs (Takeuchi, 2013), making these snow pack autotrophic communities key primary producers in glacial environments and as such, potential producers of substantial amounts of organic carbon. For example, carbon production in one Alaskan icefield attributed to snow algae alone reached 1.2 kg carbon km^{-2} (Takeuchi et al., 2006a), making snow algal communities potentially important for the global carbon cycle. Organic carbon produced by snow algal communities is also important for supporting heterotrophic bacteria within the snow pack (Takeuchi, 2013), which themselves, while not as highly studied, are important not only to the diversity of the snow pack community but also for biogeochemical cycling (Miteva, 2008; Boyd et al., 2011; Hell et al., 2013). Snow pack bacterial communities are typically dominated by *Proteobacteria*, specifically *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria,* with cell abundances ranging between $10^3 – 10^5$ cells ml^{-1} (Larose et al., 2010; Harding et al., 2011; Hell et al., 2013; Lutz et al., 2016), and several studies reporting evidence for their active cycling of nitrogen (Miteva, 2008; Amoroso et al., 2010; Fujii et al., 2010; Boyd et al., 2011; Larose et al., 2013b).

At present, research into the roles of heterotrophic and autotrophic snow pack communities on biogeochemical cycling is currently in its infancy. Of the knowledge that is available, the majority has been derived during the main growing seasons of spring and summer, with only one study addressing heterotrophic activity in a snow pack environment during polar winter (Amoroso et al., 2010). Given that snow pack environments comprise a large portion of the Earth's land surface and host abundant and active autotrophic and heterotrophic communities, further investigations into microbially mediated nutrient cycling is warranted within these systems.

1.2.1.4 Not so bare ice

Bare ice surfaces of ice sheets and glaciers have commonly been overlooked as a supraglacial environment capable of hosting life. Even though life in surface ice was documented as far back as 1872, during Adolf Erik Nordenskiöld's preliminary Greenland explorations (Nordenskiöld, 1872), research has since predominantly focused on cryoconite hole 'hot spots' (Edwards et al., 2013). Recently, however, studies have begun to focus on bare ice for two main reasons. First, due to its proportional dominance of the supraglacial landscape, with one study reporting that bare ice comprised 97.5 % of the supraglacial environment, on a 25 km east-west transect of the south-west

coast of the GrIS in August 2014, while cryoconite holes and surface water, on average, comprised only 0.6 % and 1.9 %, respectively (Ryan et al., 2018). Second, because the conspicuous presence of heavily pigmented microalgal blooms of the Mesotaeniaceae (Zygnematophyceae, Streptophyta), otherwise known as 'glacier algae', in bare ice have been found to produce blooms visible from space (Fig. 1.3) (Tedstone et al., 2017; Williamson et al., 2019).

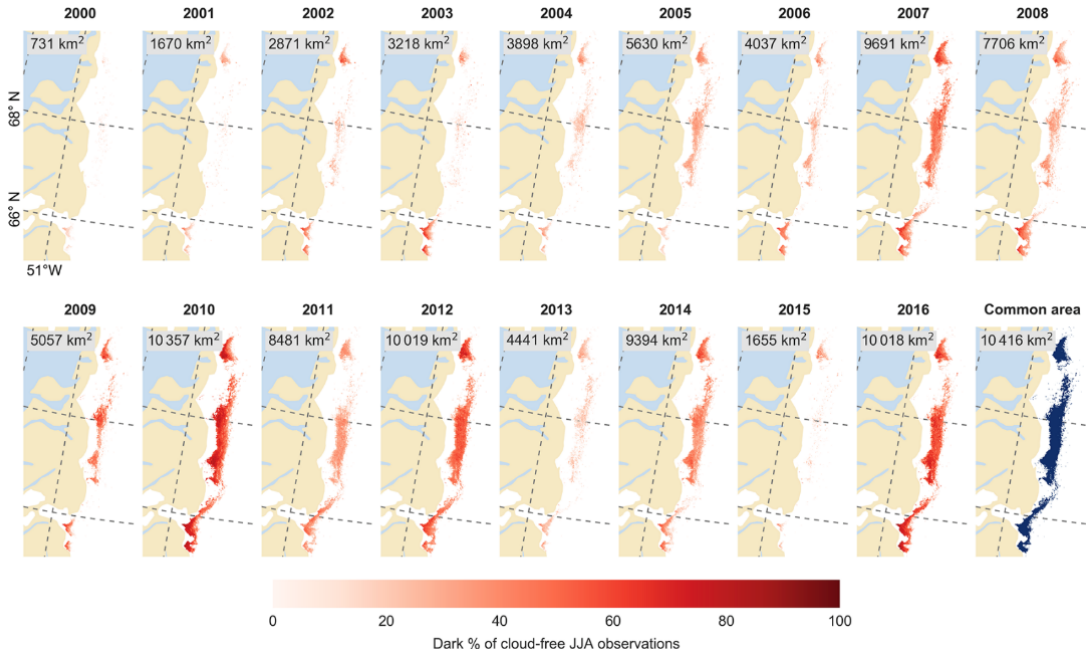

Figure 1.3: Satellite observations of darkening surface ice attributed to glacier algal blooms on the south-west GrIS during the summer (June-August) (Tedstone et al., 2017).

Blooms of glacier algae have since been documented throughout the cryosphere, including Siberia (Takeuchi et al., 2006b; Takeuchi et al., 2015; Tanaka et al., 2016), Antarctica (Ling and Seppelt, 1993), the Himalayas (Yoshimura et al., 1997), Svalbard (Remias et al., 2012a), Alaska (Takeuchi, 2001b, 2013; Ganey et al., 2017), and Greenland (Uetake et al., 2010; Yallop et al., 2012; Stibal et al., 2017a; Williamson et al., 2018) in high abundances (Williamson et al., 2019). For example, on the GrIS, cell abundances range from 9.1×10^4 to 29.5×10^4 cells ml^{-1} at the margin (Yallop et al., 2012), from <100 to 8.5×10^4 cells ml^{-1} ~ 30 km inland on the south-west coast (Stibal et al., 2017a) and from 0 to 1.6×10^4 cells ml^{-1} ~ 30 km inland from the snow ablation line in an area known as the Dark Zone (Williamson et al., 2018; Williamson et al., 2019). Given the high abundances achieved during the summer ablation season and the significant pigmentation of the glacier algae cells, blooms are likely to significantly influence chemical and physical processes in glacier and ice sheet systems, yet much remains unknown about their bloom dynamics, growth constraints or effects on biogeochemical cycling.

Glacier algae reside primarily in the top ~ 2 cm of the ice surface, commonly referred to as the weathering crust. During summer, the weathering crust receives intense solar radiation 24 hours a day and is subject to extreme temperature fluctuations, resulting in a highly dynamic and hydrologically connected environment (Irvine-Fynn et al., 2012; Cook et al., 2016a; Tedstone et al., 2020). During winter, the same environment is subject to complete darkness, sub-zero temperatures and snow pack cover. These drastic changes in conditions require glacier algae to utilize cold adaptive mechanisms such as increased concentrations of polyols and sugars (Roser et al., 1992; Chapman et al., 1994; Lyon and Mock, 2014). One unique adaptation to this high-light, low temperature environment, is their production of a unique UV-VIS absorbing pigment, purpurogallin phenolic pigment (purpurogallin carboxylic acid-6-O-β-D-glucopyranosidel (Remias et al., 2012b)), at 11-times the cellular content of chlorophyll a (Williamson et al., 2020). This "sun-screen" pigment has recently been demonstrated to protect the cells from harmful UV radiation (Yallop et al., 2012; Lutz et al., 2016; Williamson et al., 2018) whilst simultaneously repurposing captured solar energy for melt water generation proximal to the cells, providing access to liquid water and nutrients critical to life (Williamson et al., 2020). This pigmentation also makes glacier algae potent light absorbing particles (LAPs), similar to atmospheric dust or black carbon, whose presence in surface ice efficiently decreases the surface ice albedo, i.e. reflectivity, enhancing energy absorption and melt of glaciers and ice sheets (Stibal et al., 2017a; Cook et al., 2020; Tedstone et al., 2020; Williamson et al., 2020). While weathering crust dynamics have been shown to create challenges in quantifying the exact impact of glacier algae on albedo, *in-situ* observations (Yallop et al., 2012; Stibal et al., 2017a; Ryan et al., 2018; Cook et al., 2020; Williamson et al., 2020) and modelling efforts (Tedstone et al., 2017; Tedstone et al., 2020) have suggested that glacier algae are responsible for the decline in albedo in the south west of the GrIS, leading to increased surface melt. In fact, one study recently quantified an acceleration of melting by up to 26.15 ± 3.77 % in localized patches of high-biomass accumulation of algae in the surface ice of the south western GrIS, resulting in an additional 8.8 – 12.2 Gt and 4.4 – 6.0 Gt of runoff from the GrIS in 2016 and 2017, respectively (Cook et al., 2020). As the GrIS is the second largest contributor to global sea level rise (Box and Sharp, 2017; Bamber et al., 2018), it is imperative that our understanding of bloom development and dynamics is improved.

1.2.2 Biogeochemical cycles

1.2.2.1 Carbon

1.2.2.1.1 Carbon cycle

Carbon (C) is essential for all life as it is incorporated into all organic and inorganic matter existing today. The steady movement of carbon between various reservoirs in the earth is known as the carbon

cycle. Pre-human intervention, the global carbon cycle was primarily mediated by three main systems, atmospheric, terrestrial and oceanic (Post et al., 1990). Terrestrial systems, which at present contain ~ 2,000 Gt C (Falkowski et al., 2000), most commonly influence the carbon cycle via photosynthesis, removing CO_2 from the atmosphere and storing it as organic matter. This carbon is later returned to the atmosphere in several ways on differing timescales, including heterotrophic respiration of plant derived organic matter, autotrophic respiration by the plants themselves and large disturbances such as fires (Post et al., 1990; Falkowski et al., 2000). The oceanic reservoir is significantly larger than both the terrestrial and atmospheric reservoir, containing ~ 38,000 Gt C; the atmosphere containing only ~ 720 Gt C (Falkowski et al., 2000), with influences slightly more complicated than terrestrial systems due to the existence of physiochemical pathways (the solubility pump) additional to biological pathways (the biological pump).

The oceanic solubility pump describes the physio-chemical process by which carbon is transferred from the ocean's surface to its interior, and as such, its efficiency is determined by thermohaline circulation in the ocean, as well as seasonal changes in ventilation (Broecker and Peng, 1992; Stocker and Schmittner, 1997). CO_2 from the atmosphere dissolves in ocean surface waters, forming a weak carbonic acid, that later binds with carbonate anions and water to create bicarbonate. As this warm surface water is transported to the poles, it cools and sinks, storing the carbon on either short term (months to decades) or a long term timescales (centuries to millennia), depending on the depth of remineralisation, before upwelling events resurface the deep water, allowing it to re-equilibrate with the atmosphere (Passow and Carlson, 2012). The biological pump further influences oceanic carbon cycling through phytoplankton photosynthesis, which lowers the partial pressure of CO_2 in surface waters allowing further draw-down of atmospheric CO_2 (Post et al., 1990). Heterotrophic respiration then oxidizes the organic carbon as it sinks, increasing dissolved inorganic carbon concentrations in the ocean interior. Transport of organic carbon, via the biological pump, from the surface to deep water currently accounts for ~ 11 – 16 Gt C $year^{-1}$ (Falkowski et al., 2000). Additionally, some phytoplankton and zooplankton construct shells through the production of calcium carbonate, which later dissolves to some fraction at depth, furthering the transport of dissolved inorganic carbon from surface to deep ocean waters in a process known as the carbonate pump (Passow and Carlson, 2012). While lithospheric reservoirs contain the greatest amount of stored carbon on Earth, e.g. sedimentary carbonates contain > 60,000,000 Gt C (Falkowski et al., 2000), the weathering of rock, and therefore its influence on the carbon cycle, occurs on much longer (geologic) time scales as compared to terrestrial, atmospheric, and oceanic interactions, which can occur in time scales as short as hours.

1.2.2.1.2 Carbon in supraglacial environments

Understanding the potential global importance of carbon cycling in glacial systems is important to fully constrain the global carbon cycle, which in turn has consequences for the shrinking cryosphere, as atmospheric carbon levels have been linked to the rates of glacier retreat on a wide range of temporal and spatial scales (Box and Sharp, 2017). Inorganic carbon is not commonly a limiting factor to supraglacial autotrophs due to direct atmospheric interaction, with the exception of ice lidded cryoconite holes in Antarctica that display potential inorganic carbon limitation due to restricted atmospheric interaction (Tranter et al., 2004; Foreman et al., 2007). Dissolved organic carbon, however, is derived from three main sources in supraglacial environments. First, and most labile to heterotrophs, is autochthonous organic carbon produced by autotrophs (Hodson et al., 2007; Anesio et al., 2010; Stibal et al., 2012b). Second, is organic vegetation (local allochthonous organic carbon) deposited from deglaciated areas surrounding glaciers, containing more recalcitrant organic carbon, mainly in the form of lignin and cellulose (Bhatia et al., 2010; Xu et al., 2010; Stibal et al., 2012b). Third, is allochthonous organic carbon deposited via dry deposition in the form of black carbon compounds derived from soot, brown carbon compounds commonly derived from forest fires, fossil fuel combustion and other anthropogenic forces (Keegan et al., 2014; Ryan et al., 2018; Williamson et al., 2020), all of which can only be accessed by some types of supraglacial bacteria (Margesin et al., 2002; Xu et al., 2010; Stibal et al., 2012b).

Active carbon cycling has thus been documented in supraglacial environments across the cryosphere, facilitated by the excess supply of both inorganic and organic carbon (Foreman et al., 2007; Hodson et al., 2007; Stibal et al., 2008a; Anesio et al., 2009; Hodson et al., 2010c; Hodson et al., 2010b; Anesio and Laybourn-Parry, 2012; Stibal et al., 2012a; Stibal et al., 2012b). However, rates of carbon uptake and release in the cryosphere are variable due to many different physio-chemical factors that can influence microbial activity. For example, sediment thickness and organic matter content has been shown to significantly impact carbon cycling in cryoconite holes, as only holes containing < 3 mm of sediment are able to produce a net autotrophic habitat (Telling et al., 2012a). As such, reports of net ecosystem production (NEP), the balance between autotrophy and heterotrophy, in cryoconite holes across the cryosphere range from -0.01 – 0.92 μg C g^{-1} hr^{-1} (Hodson et al., 2010c; Stibal et al., 2012a; Telling et al., 2012a), with negative numbers representing net carbon sources. Knowledge of carbon cycling in snow pack environments, however, is, at present, limited. One study of a temporal snow pack reported a high algal and bacterial production rate for red snow (6295 – 9625 μg C l^{-1} hr^{-1}) (Thomas and Duval, 1995), compared to bacterial production reported for snow in the GrIS Dark Zone (0.01 μg C l^{-1} hr^{-1}) (Nicholes et al., 2019), while an NEP rate of < 42 μg C l^{-1} hr^{-1} was reported for red snow sampled from a eastern glacier on the GrIS (Lutz et al., 2014). A comprehensive review

of carbon cycling rates in supraglacial environments around the world reported a NEP range of -3 to 9 kg C km^{-2} d^{-1}, further supporting the variability in these environments (Stibal et al., 2012b).

Large ice sheet interiors are commonly net autotrophic (carbon sinks), whereas small glaciers and ice sheet margins tend to be net heterotrophic (carbon sources) (Stibal et al., 2012b). NEP rates recently reported for surface ice dominated by glacier algae corroborate this, with NEP ~ 1.25 mg C l^{-1} d^{-1} (Williamson et al., 2018). Yet, Yallop et al., (2012) reported a surprising 30:1 ratio of glacier algal to bacterial production rates in surface ice dominated by glacier algae coverage, which was later supported by Nicholes et al., (2019). These data suggest an inefficient microbial remineralization within surface ice dominated by glacier algae, likely leading to these areas becoming locations of extreme organic carbon accumulation (Yallop et al., 2012). This was later confirmed by Williamson et al., (2018) who estimated an average carbon accumulation of ~ 15.82 ± 8.14 kg C km^2 for a large glacier algal bloom occuring on the GrIS during 2016, with net production (NP) of glacier algal communities reaching 50 kg C km^2 in some regions of the Dark Zone of the GrIS as compared to ~ 1.37 kg C km^2 estimated for cryoconite holes in the same region (Cook et al., 2012). Glacier algal blooms are thus highly likely to contribute significantly more to organic carbon production within supraglacial environments than microbial communities in cryoconite holes (Cook et al., 2012). Given that transport of dissolved organic carbon from supraglacial environments is highly likely (Hood et al., 2009; Yallop et al., 2012; Stibal et al., 2012b; Musilova et al., 2016; Musilova et al., 2017), significant production of autochthonous organic carbon driven by glacial algal blooms may have important impacts to downstream ecosystems, with several studies already reporting glacial runoff containing notable concentrations of supraglacial particulate and dissolved organic matter (Barker et al., 2006; Bhatia et al., 2010), yet further work into quantifying *in-situ* dissolved organic carbon abundance for ice populated by glacier algae and its export to downstream environments is required. As global temperatures continue to rise, snow pack coverage on glaciers and ice sheets will decrease, leading to greater bare ice surface area and an extended growing season available for glacier algal blooms, with consequent increases in bloom magnitude and extent predicted into the future (Williamson et al., 2020). This may drive elevated production of organic carbon, with implications for downstream ecosystems.

1.2.2.2 Nitrogen

1.2.2.2.1 Nitrogen cycle

Nitrogen (N) is an essential biomolecule required by all living things and is responsible for many bio-critical processes such as chlorophyll and amino acid production in primary producers (Arrigo, 2005; Canfield et al., 2010). It is also the second most abundant element by dry weight of organisms after

carbon (Canfield et al., 2010). N is highly abundant on the surface of the earth, with ~ 5 billion metric tons contained within the atmosphere, ocean, biota, sedimentary rocks and soil organic matter, yet < 2 % is accessible to organisms (Mackenzie, 1994). The remaining 98 % is retained within sedimentary rocks (20 %) or as di-nitrogen gas (N_2) in the atmosphere (78 %) (Galloway, 1998). The latter is commonly accessed by the biosphere through fixation from the atmosphere by diazotrophic microorganisms in an energy taxing process that breaks the triple bond and converts N_2 into a reactive form of N, commonly attached to carbon, hydrogen or oxygen, which is subsequently bio-available to organisms. This microbially mediated nitrogen fixation is a vital process of N cycling, accounting for 90 – 103 Tg N yr^{-1} (Galloway et al., 1995), yet it will only be performed if other, more easily available N is lacking given the energetic demands (Gutschick, 1978).

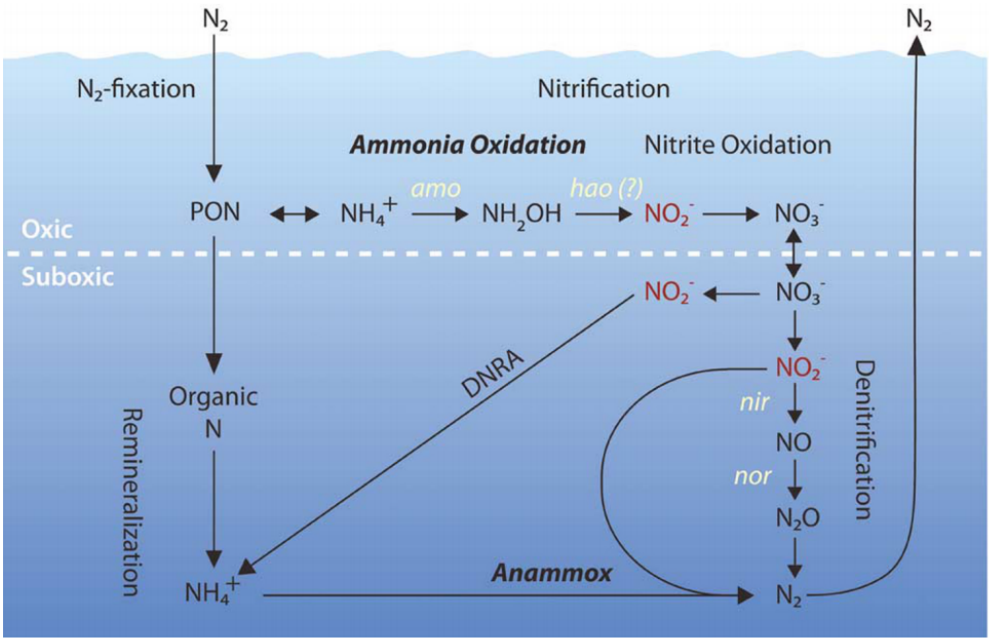

Figure 1.4: Schematic of nitrogen species and transformations a marine environment (Francis et al., 2007).

N exists in many forms, yet is almost exclusively cycled by microbiological processes (Fig. 1.4) (Vitousek et al., 2002; Francis et al., 2007). Additional to nitrogen fixation, microbes also perform nitrification, a process which oxidises ammonium into nitrite (NO_2^-), then nitrate (NO_3^-) (Fig. 1.4). Nitrification is also extremely important to the nitrogen cycle and highly beneficial to microorganisms, as bacteria utilize the energy released, while autotrophs uptake the NO_3^- produced. It is also the only substantial process known to produce NO_3^-, with terrestrial and marine ecosystems producing on the order of 90 – 130 and 40 – 200 Tg N yr^{-1}, respectively, as the alternative pathways that include rock weathering and lightning strikes produce comparatively smaller yields, on the order of 3 – 5 Tg N yr^{-1} (Galloway et al., 1995; Noxon, 1976; Holloway et al., 1998; Holloway and Dahlgren, 2002). Remineralization of ammonium from organic matter allows the production of

available inorganic N to begin again. In anoxic conditions, such as oxygen minimum zones within the marine environment, NO_3^- may be reduced back to NO_2^-, which can be converted back to the gaseous phase in the form of N_2O or N_2 by denitrification or NH_4^+ may be oxidized, at the expense of NO_3^- or NO_2^-, back to N_2 by anammox reactions, as more recently discovered (Mulder et al., 1995).

1.2.2.2.2 Nitrogen in supraglacial environments

N in supraglacial environments is sourced from three main inputs, snow melt, rain fall and ice melt (Hodson et al., 2005; Telling et al., 2011; Telling et al., 2012b), yet, contributions from each source are highly heterogenous. For example, on Svalbard valley glaciers during the 1999/2000 season, snow melt and rainfall comprised 99.1 % of the total nitrogen (TN) input, with ice melt accounting for only 0.34 % (Hodson et al., 2005). However, N input to supraglacial environments via rainfall has been found to fluctuate greatly between years and even within seasons due to anthropogenic pollution (Hodson et al., 2009), and as such, snow melt is commonly considered the dominant N source within these systems. This is supported by total inorganic nitrogen (TIN) concentrations in supraglacial environments typically show a decreasing linear trend since the time since snowline retreat (Hodson et al., 2005; Telling et al., 2011; Telling et al., 2012b; Stibal et al., 2012a).

Microbial activity has also been shown to strongly influence nitrogen speciation and cycling within supraglacial environments. Within cryoconite holes, for example, cyanobacteria perform nitrogen fixation, facilitating subsequent nitrification by heterotrophic bacteria (Hodson et al., 2005; Wynn et al., 2007; Telling et al., 2011; Cameron et al., 2012; Telling et al., 2012b). On valley glaciers in Svalbard, Telling et al., (2011) demonstrated active nitrogen fixation within cryoconite holes at the mid to late ablation season, once the main snow pack had ablated. The presence of a strong negative correlation between nitrogen fixation and both DIN and total inorganic nitrogen (TIN) concentrations, further suggested that inorganic N concentration was a first order control on processes of nitrogen fixation, and thus the zone of active fixation and organic matter remineralization within supraglacial systems is likely to progress upslope as the snowline retreats and removes the steady supply of bioavailable nitrogen (Telling et al., 2011). These relationships were later corroborated by studies of cryoconite holes on the GrIS on a transect traversing the Dark Zone, an annually recurring area of decreasing surface ice albedo, first mentioned in the literature in the early 1990's (Oerlemans and Vugts, 1993; Telling et al., 2012b; Stibal et al., 2012a). In Antarctica, reports of high inorganic to organic ratios in cryoconite holes imply that organic matter recycling plays a larger role than N fixation, though this could be largely due to the ice-lidding that occurs in Antarctic environments (Tranter et al., 2004).

To date, studies of microbial communities in snow pack environments, though limited, have clearly

shown a significant effect on N cycling within these systems by microbial activity (Fujii et al., 2010; Amoroso et al., 2010; Boyd et al., 2011; Hell et al., 2013; Larose et al., 2013b). For example, Larose et al., (2013) found that assimilation was likely a major pathway for N within the microbial communities and that nitrite and nitrate ammonification had the highest reads for gene expression, suggesting that N might even be limiting for these environments. Another study reported the presence of *nif*H genes in supraglacial snow environments, suggesting the potential for N fixation (Boyd et al., 2011). Further investigations into the influence of snow microbial communities on N cycling is necessary to constrain the potential importance for on global cycles of N, particularly considering the extent of Earth's snow cover and its effect on downstream ecosystems.

N cycling in bare ice surface environments of glaciers and ice sheets has yet to be investigated. To date, only two studies have quantified nitrogen concentrations in surface ice of the GrIS Dark Zone, but both appear to focus on dispersed cryoconite as opposed to ice containing Streptophyte glacier algae (Telling et al., 2012b; Wadham et al., 2016). Yallop et al., (2012) was the first to study glacier algae and their potential impact on carbon and nutrient cycling by quantifying a 30:1 ratio of primary production to bacterial production for surface ice with dense algal coverage 2 km inland from the GrIS margin, which was significantly lower than the ~ 11:1 ratio reported for cryoconite holes (Stibal et al., 2012a), suggesting that these environments could become areas of high organic carbon accumulation. A similar 30:1 ratio was also reported for surface ice in the Dark Zone (Nicholes et al., 2019), confirming that bare surface ice inhabited by glacier algae could have vastly different nutrient cycling compared to cryoconite holes. Further studies regarding N abundance and cycling in surface ice dominated by glacier algae blooms are vital for advancing the current understanding of bloom dynamics and their potential importance for global N cycling. The more we understand about how they thrive in these environments the better we can predict their likely expanse in the current warming Arctic. As snow packs retreat further and earlier, with increased melt, bare ice exposure increases in concert and for prolonged periods, resulting in a greater area for bloom development and elevated nutrients and liquid water released from the ablating ice (Cook et al., 2020).

One factor with the potential to change N cycling in all supraglacial environments is the increased burning of fossil fuels, which introduces N oxides into the atmosphere, that can be carried around the world and deposited onto glacier and ice sheet surfaces by wet and dry deposition (Hodson et al., 2005; Telling et al., 2011). As anthropogenic forces continue to rise it is inevitable that N deposition will follow suit over the cryosphere. NO_3^- and NH_4^+ deposition has already increased by 65 % and 20 %, respectively, on Svalbard valley glaciers since preindustrial times (Kekonen et al., 2005), accounting for 23 % (mean of 1998/1999 and 1999/2000 data) or 19.5 % (1999/2000 data only) of TN inputs, respectively (Hodson et al., 2005; Telling et al., 2011). NO_3^- deposition on some Alpine and

central Asian glaciers and the GrIS has increased by 90 %, 130 % and 100% respectively (Döscher et al., 1995; Fischer et al., 1998; Olivier et al., 2006), while NH_4^+ deposition rates on Himalayan and Alpine glaciers have increased by 100 % and 200 %, respectively (Döscher et al., 1996; Kang et al., 2002). This could have major impacts on N cycling in supraglacial environments as Telling et al., (2011) has already shown a link between the presence of TIN availability and N fixation rates in cryoconite holes. With more bioavailable N supplied to these microbial communities, energy previously required for N fixation may be used on community growth, which could have major implications for areas such as the Dark Zone as glacier algae have already been reported to significantly impact surface albedo and thus melt rates (Takeuchi, 2013; Takeuchi et al., 2015; Cook et al., 2017b; Tedstone et al., 2017; Cook et al., 2020; Tedstone et al., 2020).

1.2.2.3 Phosphorus

1.2.2.3.1 Phosphorus cycle

Phosphorus (P) is the third major macronutrient required by all living organisms for vital cellular processes. Organisms use phosphorus for many different biological pathways, for example the production of nucleic acids, phospholipids and many other compounds. In contrast to carbon and N, P is comparatively limited in bioavailable forms, with a lack of gaseous phase, precluding fixation from the atmosphere (Schindler, 1977). In fact, P is primarily found in only one dissolved inorganic form, orthophosphate (PO_4^{3-}), resulting in low or undetectable concentrations in most aquatic environments (Karl, 2000; Paytan and McLaughlin, 2007), with P consequently identified as the key limiting nutrient for primary production in many aquatic environments (Dodds, 1993; Filippelli and Delaney, 1994; Ruttenberg, 2003; Elser et al., 2007; Filippelli, 2008). P is principally a rock derived mineral and is only released into aquatic systems via physical or chemical weathering of P rich rocks such as apatite. Dissolved P also has a high affinity for binding to sediments (Sommer and Lampert, 1997), and accordingly, 90 % of riverine deposits to oceans are in the particulate P form (PP) (Ruttenberg, 2003). P occurs in a variety of organic compounds, which are often found in the dissolved phase (i.e., dissolved organic phosphorus (DOP)). Organisms have thus developed phosphatase enzymes as a response to low DIP concentrations, which allow the cleavage of DOP molecules to liberate P that is subsequently bioavailable (Dodds, 2002; Stibal et al., 2009). The presence of these enzymes is thus an indicator of low DIP abundance (Cembella et al., 1982; Jansson et al., 1988; Dodds, 2002; Stibal et al., 2009).

1.2.2.3.2 Phosphorus in supraglacial environments

Presently, there are limited studies of dissolved P abundance and cycling in supraglacial environments. Most P in glacial environments is reported as sediment bound, with dissolved

inorganic concentrations typically low or below reasonable detection limits (Hodson et al., 2005; Hawkings et al., 2016). P deposition to supraglacial environments is variable, and includes rock debris from surrounding deglaciated areas, windblown deposits and melt out of particles from meteoric ice (Stibal et al., 2008b; Wientjes et al., 2011). Most present literature focuses on cryoconite holes to investigate P cycling in supraglacial environments, similar to carbon and N, and several studies have determined P to be the limiting nutrient for primary production in cryoconite holes, akin to other aquatic environments (Mindl et al., 2007; Säwström et al., 2007; Stibal and Tranter, 2007). Yet, these investigations remain limited, with the greatest insight into bioavailable P and P cycling in cryoconite holes provided by only two studies (Stibal et al., 2008b; Stibal et al., 2009).

Several studies have reported connections between supraglacial microbial communities and particulate abundance, likely as a result of particulates supplying necessary nutrients such as P (Grzesiak et al., 2015; Stibal et al., 2015; Stibal et al., 2017a). Speciation and potential bioavailability of P on a high Arctic glacier was investigated by Stibal et al., (2008) who determined a potential bioavailable particulate inorganic P pool of ~ 0.16 mg P g^{-1} in cryoconite debris, a surplus, in fact, for cryoconite hole microbial communities that only required ~ 1.2 % of the total bioavailable particulate inorganic P (~ 2 μg P g^{-1}). This study did not, however, investigate the ability of the microbial community to access this bioavailable particulate P, and in a later study suggested that severe P limitation in cryoconite holes, was indicated by the rate of phosphatase activity apparent, suggesting the previously determined pool of potentially bioavailable P may be inaccessible to some cells (Stibal et al., 2009).

Organic P, in addition to particulate inorganic P, has also been suggested to play an important role in P cycling in supraglacial environments. Stibal et al., (2008) argued that the DOP pool in supraglacial environments had long been overlooked as an important factor in nutrient fluxes, as DOP concentrations reportedly exceeded those of DIP 4-fold for all glacial water samples. Further, several studies have investigated phosphatase activity in glacial environments throughout the Arctic and Antarctic, reporting similar rates (~ 20 – 50 nmol l^{-1} h^{-1}) for Antarctic cryoconite holes, ice covered lakes and subglacial discharge (Dore and Priscu, 2001; Mikucki et al., 2004; Foreman et al., 2007), and rates ~ 2 – 6 times higher for Arctic cryoconite holes (Stibal et al., 2009), indicating that both DOP and particulate organic P may be vital for otherwise P limited environments.

At present, investigations into P abundance or cycling within the GrIS Dark Zone are limited. To date, Telling et al., (2012) is the only study to report DIP concentrations for surface ice in this location, with all concentrations below the detection limit (0.33μM P), and the surface ice sampled described as ice containing dispersed cryoconite, as opposed to glacier algae. A link between the presence of particulates, e.g. ablating ancient Holocene dust, and the extent of glacier algae blooms in

the Dark Zone has also been suggested using satellite and modelling methods (Tedstone et al., 2017), while another study reported that the presence of dust explained 9.5 % of algal abundance and biovolume variation in their study of the same location (Stibal et al., 2017a). A significant relationship between dust content and microbial abundance was also found for GrIS surface ice environments (Stibal et al., 2015). Further studies into potential particulate and organic P nutrient abundance in surface ice habitats and the potential for microbial communities to access these pools are clearly necessary to fully constrain P cycling within supraglacial environments.

1.2.3 Snow pack as a nutrient source

1.2.3.1 Snow pack formation and influence of atmospheric chemistry

Snow packs in supraglacial environments, i.e. snow that accumulates annually in the winter and melts in the spring, play a variety of important roles in Arctic environments. For example, they provide insight into atmospheric conditions, provide insulation for environments below, act as unique habitats harbouring diverse microbial communities and impact glacial and ice sheet hydrology during the ablation season (Kuhn, 2001; Larose et al., 2013a). Snow packs also act as an important storage vessel for nutrients during winter, which are then exported to underlying surface ice and downstream ecosystems during spring melt (Kuhn, 2001; Wynn et al., 2007; Lutz et al., 2014). Nutrients are typically deposited to snow packs via two main pathways; wet deposition (snowfall and rainfall) and dry deposition. Snow formation occurs in the atmosphere as particles of ice grow from vapor deposition at temperatures below 0 ºC. However, at temperatures above -40 ºC ice formation is not spontaneous, thus most snow crystals use a substrate to initiate the formation, with vapor condensing around the substrate (Larose et al., 2013a). Potential substrates are extremely varied and can include dust, sea salt particles, sulphate, microbes and combustion products from industrial plants and volcanoes (Jones, 2001; Kuhn, 2001; Christner et al., 2008; Domine and Shepson, 2002). Snow crystals also scavenge trace gasses and nutrients, which are incorporated into the crystal lattice during formation in the atmosphere (Fig. 1.5) (Kuhn, 2001; Domine and Shepson, 2002; Larose et al., 2013a). Rain also forms in the atmosphere, making it another important vehicle for transporting atmospheric particles and nutrients to snow packs. Although, rain water is denser then snow, allowing it to percolate through the snow pack, transporting nutrients and particles deeper than fresh snow (Tranter et al., 1992; Kuhn, 2001). One study also found rain water to leach solutes from the snow pack in a similar way to meltwater, furthering its impact on nutrient transport throughout snow packs (Tranter et al., 1992). Wet deposition may increase in importance in the current warming Arctic, with one study already reporting increased rainfall over Greenland driven by warm cyclone-driven events (Oltmanns et al., 2019), as elevated levels of precipitation will likely lead to greater scavenging of nutrients and anthropogenic pollutants from the atmosphere (Larose et al., 2013a).

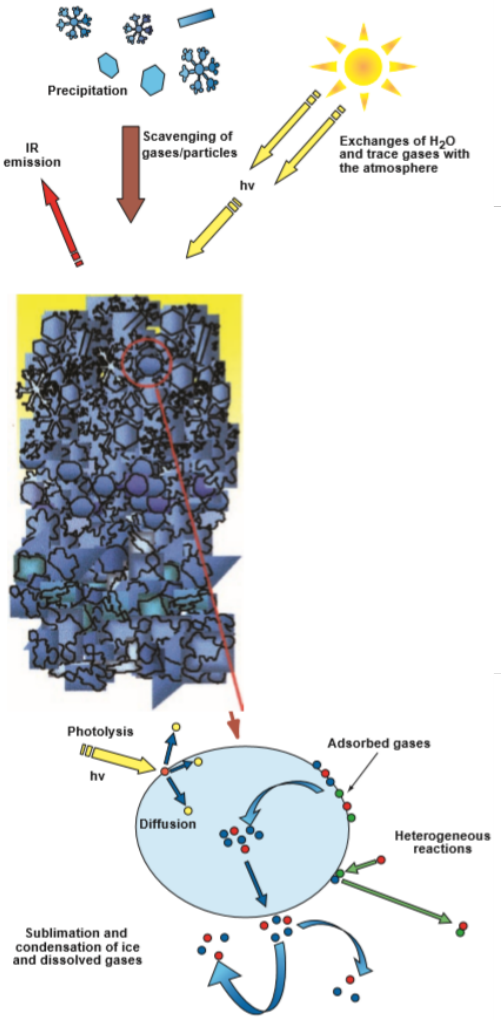

Figure 1.5: Physical and chemical processes involved in the formation and metamorphism of snow packs (Domine and Shepson, 2002).

Dry deposition occurs when particles and gases from the atmosphere are directly deposited onto the snow pack surface without scavenging by precipitation (Kuhn, 2001; Larose et al., 2013a), resulting in deposition concentrations typically proportional to the atmospheric concentration of the deposited species. Atmospheric concentrations also influence wet deposition, with seasonal fluxes of nutrients in the atmosphere influencing concentrations in the snow pack. For example, winter snow deposition typically has low, stable concentrations, while atmospheric convection in the spring leads to a spike in nutrient concentration, regardless of species (Kuhn, 2001), with snow pack layers consequently containing differing concentrations of major ions based on their respective accumulation periods (Fig. 1.6).

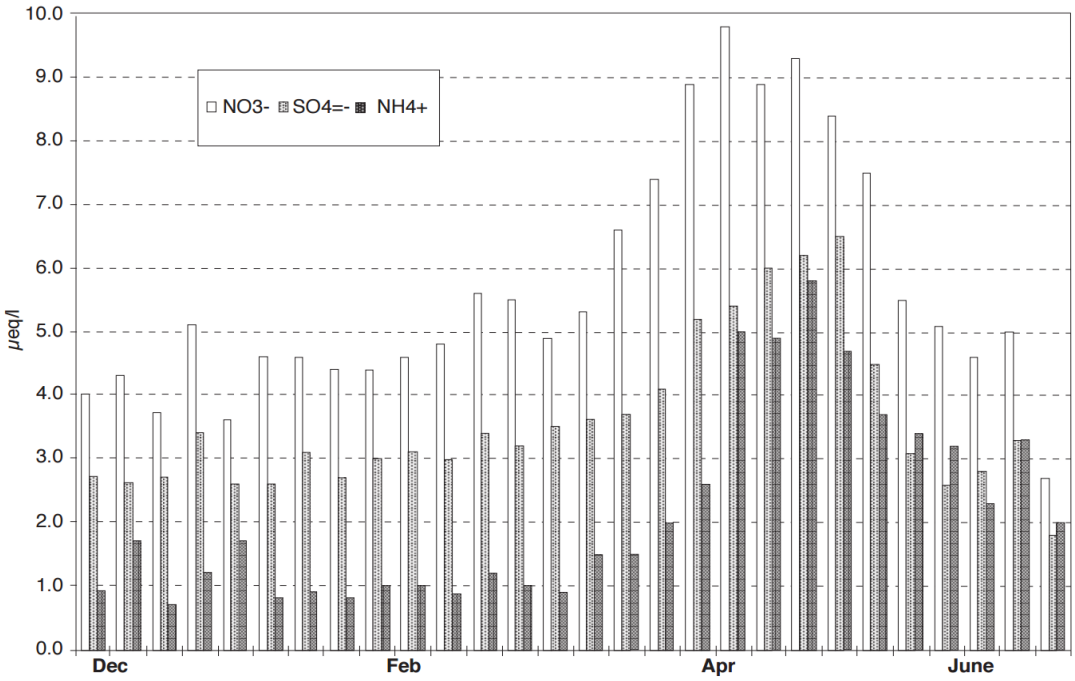

Figure 1.6: Seasonal variation in average snowpack concentrations of sulphate (SO_4^{2-}), nitrate (NO_3^-), and ammonium (NH_4^+) (Kuhn, M., 2001, Kuhn, et. al. 1998).

1.2.3.2 Redistribution of solutes

Nutrients within a snow pack are not static, with movement and redistribution of solutes a common process. Post deposition, snow crystals and solutes undergo loss and redistribution facilitated by three main physical and chemical processes; volatilization, meltwater and degradation. Gases, and thus trace nutrients, deposited via dry deposition enter the pore space between crystals in the snow pack, and are then transported throughout different layers via diffusion along concentration gradients (Larose et al., 2013a). This movement is highly influenced by snow pack physical-chemical properties such as the geometry of pore spaces and wind and vapor pressure gradients (Kuhn, 2001; Domine and Shepson, 2002; Larose et al., 2013a). A well-documented example of volatilization within snow pack environments is photochemical cycling of reactive N species, specifically photolysis of nitrate (Fig. 1.5), which has been documented in many locations in the Arctic (Honrath et al., 1999; Bock and Jacobi, 2010; Anastasio and Chu, 2009). Volatilization of solutes from the snow pack is not only important for considering changes in snow pack concentrations, but also for overlying atmospheric concentrations, which have been found to be significantly impacted by snow pack nutrient cycling (Honrath et al., 1999; Zhou et al., 2001; Amoroso et al., 2010).

Meltwater plays a key role in snow pack metamorphism, redistribution and eventual loss of solutes. Post deposition, snow crystal metamorphism is typically initiated by temperature gradients and a thin liquid boundary layer, causing molecules to diffuse from convex to concave, promoting the

distillation onto larger grains in proximity (Larose et al., 2013a). Impurities and insoluble ions are thus removed from the snow crystal interior and concentrate on the exterior and in pore spaces during this process (Fig. 1.5) (Kuhn, 2001; Larose et al., 2013a). As melt-freeze events occur throughout the accumulation season, meltwater percolating from the top of the snow pack collects the insoluble ions and impurities as it moves through the pore space (Kuhn, 2001). If conditions change and melting stops, this highly concentrated solute meltwater forms ice layers within the snow pack, which can become increasingly concentrated throughout the season with continued melt-freeze cycles (Kuhn, 2001; Meyer et al., 2006).

Additional to degradation pathways of solutes within snow packs, microbial communities should also be recognized as a potentially important route for the redistribution and modification of nutrient species. Whilst snow packs are now commonly accepted as hosting diverse and abundant microbial communities (see above section 1.2.1.3), our understanding of their influence on nutrient cycling remains largely in its infancy. Few reports have investigated microbially mediated nutrient cycling within snow packs (Segawa et al., 2005; Amoroso et al., 2010; Fujii et al., 2010; Larose et al., 2013b; Takeuchi, 2013), finding significant influences on nutrient degradation and distribution. For example, Larose et al., (2013) investigated N cycling in an Arctic snow pack and reported high genetic potential of microbial communities for nitrate and nitrite ammonification pathways, with denitrification pathways also common. They concluded that N cycling occurred primarily at the base of the snow pack, causing certain species of N to accumulate in these areas of high microbial activity. Constraining the influence of microbial communities on the distribution and solubility of macronutrients within snow packs is critical to understanding supraglacial biogeochemical cycles and potential export to downstream environments.

1.2.3.3 Preferential elution

A heterogeneous solute rich meltwater is released from the snow pack at the onset of the main melt season, as a result of metamorphism and melt-freeze cycling, containing as much as 50-80 % of the total solutes in the initial 30 % of snow melt (Johannessen et al., 1975;Johannessen and Henriksen, 1978;Cragin et al., 1996). This event, commonly referred to as the "Ionic Pulse", can have important impacts on the supraglacial environment. Certain ions are eluted from the snow pack earlier and in higher concentrations then others during the Ionic Pulse (Davies et al., 1982;Hewitt et al., 1991;Harrington and Bales, 1998) due to differing solubility of ions in snow. Ions with lower levels of solubility will be excluded from the snow crystal lattice and concentrate on the exterior, while more soluble ions will move into the interior of the lattice (Cragin et al., 1996), leading to less soluble ions eluting first at the onset of snow melt, having a higher concentration than the bulk snow pack concentration due to the lack of dilution during the initial melt (Fig. 1.7) (Cragin et al., 1996).

Insoluble ion concentrations then rapidly decrease and reach an equilibrium after the initial eluate, whereas soluble ions rarely exhibit a notable change in concentration over the melt season (Cragin et al., 1996).

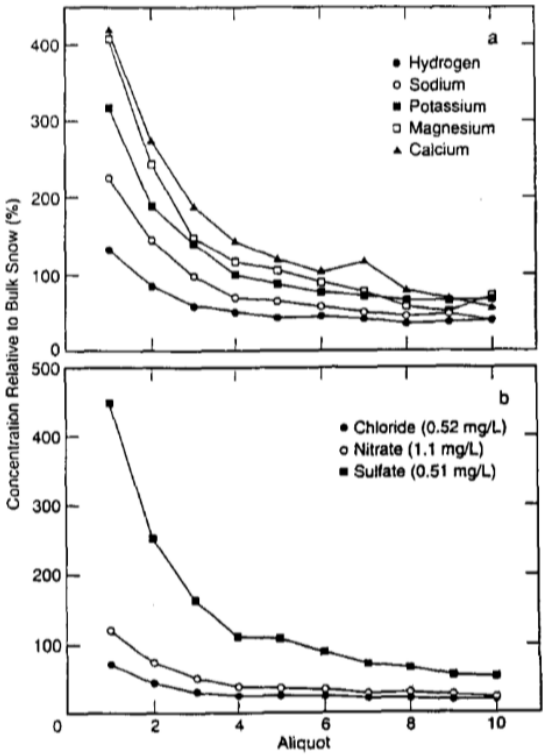

Figure 1.7: Concentrations of a) cations and b) anions from a laboratory experiment exhibiting preferential elution for natural snow (Cragin et al., 1996).

The main importance of the Ionic Pulse is that it may be a stimulant for microbial activity after the winter season (Telling et al., 2014), yet the knowledge of its importance to supraglacial microbial communities remains limited. At present only one study has investigated the impact of an Ionic Pulse on Antarctic cryoconite hole habitats, finding that it provided cryoconite holes with PO_4^{3-} and NO_3^-, nutrients which typically limit the system, leading to a spike in bacterial production as the requirement for metabolically expensive processes such as nitrogen fixation was removed (Telling et al., 2014). Other studies have only begun to link biomass and abundance of surface ice communities to snow pack presence or time since the onset of ablation. For example, glacier algae in alpine regions have been reported to decrease in biomass with increasing altitude (Yoshimura et al., 1997; Takeuchi and Kohshima, 2004; Takeuchi et al., 2009), similar to the GrIS where they have been found to decrease in biomass with distance from the margin (Williamson et al., 2018), both suggesting that time since snow pack retreat and potential nutrient input from surrounding terrestrial environments to glacier environments aid glacier algae development (Yoshimura et al., 1997; Williamson et al., 2019). As initial studies indicate links between snow packs and supraglacial

microbial communities, further investigations are required to better constrain nutrient concentrations released from snow melt during early ablation, as it likely influences supraglacial microbial community initiation and development.

1.2.4 Summary and research gaps

Despite our knowledge of microbial life in glacial environments since the Greek Aristotle, our understanding regarding the effect of these harsh environments on the microbial communities and in turn, their effect on these environments remains in its infancy. Within the past two decades research surrounding glacial microbial communities has drastically increased, due largely to their discovered impact on nutrient export from subglacial environments and decreasing surface albedo in supraglacial environments, both of which have wider implications, such as stimulation of coastal productivity and increased melt rates of areas such as the GrIS. Yet, even considering the recent increase in research there are a number of gaps that still exist in the literature. For example, only a small number of studies have investigated microbially mediated nutrient cycling in snow pack habitats and at present no studies have investigated nutrient cycling in bare ice inhabited by glacier algae. Glacier algae have recently been shown to significantly impact surface albedo on the GrIS, leading to notable increases in melt rates and runoff, thus having serious implications for global sea level rise. Only by bettering our understanding of nutrient input and abundance in supraglacial environments as well as the impact of microbial communities, such as glacier algae, on nutrient cycling in these environments will we be able to better predict their potential expansion into other areas of the GrIS and throughout the cryosphere.

Snow pack habitats cover a large expanse of the Earth's surface and are integral components of cryospheric environments. Knowledge regarding microbial communities within snow pack habitats has largely advanced in recent years, as species have been identified and compared from most areas of the cryosphere. Yet, research of microbially mediated nutrient cycling remains largely in its infancy, with only a handful of studies investigating carbon and nutrient cycling within snow packs. Further, all knowledge of microbially mediated nutrient cycling, save one study, has been derived from the main growth season of spring and summer. At present, the polar winter is considered to be a biologically inert season, due to the subfreezing temperatures, 24 hr darkness and low liquid water availability. Yet, the one study to date, which investigated microbial activity during the polar winter reported active communities capable of significantly influencing nitrogen cycling. As the polar winter comprises nearly half of the year, it is vital to better understand any potential microbially mediated nutrient cycling occurring (Chapter 2), particularly as snow packs are well known for being critical nutrient inputs to other environments during spring thaw.

Measurements of early melt season *in-situ* snow pack nutrient concentrations are largely missing from the literature as well. While several studies have indicated a significant relationship between time since snow line retreat and the activity and development of microbial communities in supraglacial environments, very few, have measured nutrient concentrations released from *in-situ* snow packs. To date no data have been reported for nutrient inputs during spring thaw to supraglacial environments in the GrIS Dark Zone, even though a significant correlation has been reported for time since snow line retreat and glacier algae biomass. Given the importance of glacier algae on surface albedo and melt rates, the lack of information regarding nutrient inputs that stimulate their growth each year is a critical gap in the literature. A comprehensive dataset of snow and ice melt nutrient concentrations during the onset of the ablation season would shed light on the different nutrient inputs to these supraglacial environments (Chapter 3). The data would provide the first quantification of nutrient inputs in this area, as well as highlight the likely speciation and range of nutrient concentrations that stimulate the development of glacier algal blooms.

Over the past two decades studies have now identified glacier algae throughout the cryosphere, yet much still remains unknown regarding their ability to thrive in bare ice habitats. Recent studies have focused on their unique pigmentation and photophysiology, as their adaptation to survive the harsh UV radiation of supraglacial environments has caused them to be a light absorbing particle capable of significantly reducing surface ice albedo, most notably in the GrIS Dark Zone. Only a handful of studies have reported nutrient concentrations from ice in the GrIS Dark Zone, yet all sampled ice containing dispersed cryoconite as opposed to glacier algae. Thus, there remains a gap in the literature regarding nutrient abundance and cycling occurring in this area, and how large scale glacier algal blooms impact these processes. A comprehensive dataset of surface ice containing the full range of glacier algae abundances present would allow for the first insight into nutrient cycling occurring within the bloom and shed light on the influence of differing glacier algae abundance on surface ice habitats (Chapter 4).

The principal aims and objectives of this thesis indented to fill some of the remaining knowledge gaps regarding nutrient input, abundance and cycling in supraglacial environments. Further, the temporal transitions of the datasets allow for the examination of connections between seasons, such as how microbially mediated nutrient cycling occurring during the polar winter may have knock on effects, which impact glacier algal blooms during the main growth season. While study constraints required that the experiment investigating nutrient cycling in polar winter (Chapter 2) utilized snow collected in Svalbard as opposed to Greenland, which is the main study sight of the remaining thesis (Chapters 3 & 4), studies of snow pack microbial communities from across the Arctic report similar dominating

genera. Thus, an assumption is made that the heterotrophic communities found in snow from Svalbard are similar to those found in Greenland and would therefore respond comparably.

1.3 Aims and Objectives

1.3.1 Primary Research Aim

Collect and synthesise a dataset of snow and surface ice nutrient concentrations from supraglacial environments to constrain key biogeochemical cycles of carbon and macronutrients occurring in these glacial environments.

1.3.2 Objective 1 (Chapter 2)

Investigate heterotrophic community response to the addition of critical macronutrients in a simulated cold, dark Svalbard snow pack environment, in turn assessing microbially mediated nutrient cycling during polar winter and the availability of particulate phosphorus to snow pack heterotrophic communities.

The polar winter has been considered a season of minimal biogeochemical importance due to the 24 hr darkness, subfreezing temperatures and extremely limited liquid water abundance. As a result, most studies investigating biogeochemical cycles, particularly microbially mediated nutrient cycling, focus on the main growth seasons of spring and summer. To date, only one study has investigated microbial activity during the polar winter, revealing that microbially mediated N cycling was not only occurring, but at levels high enough to influence overlying atmospheric N concentrations (Amoroso et al., 2010).

Dry and wet deposition commonly deliver particulate nutrients to snow pack environments throughout the accumulation season (Kuhn, 2001; Domine and Shepson, 2002), yet little to no investigations have been made into their influence as a potential nutrient source. Links have been drawn between particulates and microbial abundance and diversity (Grzesiak et al., 2015; Stibal et al., 2015; Stibal et al., 2017a) while another attempted to quantify bioavailable fractions of P in cryoconite material (Stibal et al., 2008b), yet no studies to date have investigated possible associations between snow pack microbial communities and particles, particularly the potential for the particles to act as a nutrient source. As snow pack microbial communities are hypothesized to seed underlying surface ice environments (Musilova et al., 2015), this has important applications for other supraglacial environments. If microbial communities in snow pack environments are active during the polar winter and influencing nutrient cycling, then snow packs are not the static geochemical reservoirs

previously considered, with important implications for supraglacial and downstream environments (Kuhn, 2001; Telling et al., 2012b; Larose et al., 2013a; Telling et al., 2014).

In order to address this uncertainty, a 5-week incubation experiment will be conducted in a cold (5 °C), dark environment using previously sampled snow from Ny-Ålesund, Svalbard incubated under different dissolved and particulate nutrient treatments. Weekly destructive sampling will allow for a temporal analysis of changes in nutrient abundance and microbial abundance and diversity in response to treatments. Particulate phosphorus extractions will also be conducted on the particulates added to the incubations allowing for furthered insight into the potential accessing of particulate P by microbes in a snow pack environment. These data will assist in addressing the knowledge gap in snow pack microbially mediated nutrient cycling as well as community dynamics during the polar winter.

1.3.3 Objective 2 (Chapter 3)

Quantify nutrient inputs from a seasonal snow pack and ablating ice to supraglacial environments of the Greenland ice sheet Dark Zone, and their importance for the initiation of glacial algal blooms.

Blooms of Steptophyte 'glacier algae' have recently been reported as a significant factor driving a reduction in albedo, and consequently an increase in surface melt, of the GrIS, particularly along the western ice sheet margin commonly referred to as the Dark Zone, given their widespread presence and abundance achieved in the ice surface during summer ablation seasons (Stibal et al., 2012a; Yallop et al., 2012; Tedstone et al., 2017; Stibal et al., 2017a; Williamson et al., 2018). As the GrIS has experienced a significant net mass loss over the past two decades (Rignot and Kanagaratnam, 2006; Rignot et al., 2011; Shepherd et al., 2012), driven predominantly by an increase in surface melt (~ 68 % of total ice mass loss; Enderlin et al., 2014), furthering knowledge on bloom development is critical. At present, much remains unknown about the factors that promote bloom initiation at the onset of melt. However, snow packs are known to deliver key nutrients to supraglacial environments as the ablation proceeds (Kuhn, 2001; Wynn et al., 2007; Telling et al., 2012b; Telling et al., 2014; Larose et al., 2013a), and ice melt has also been found to input significant N to the GrIS supraglacial system (Telling et al., 2012b). It is therefore likely that both snow and ice ablation provide important nutrient inputs to the GrIS supraglacial environment that may be critical for the initiation and subsequent development of glacial algal blooms. To date, however, this knowledge is completely lacking.

This objective and current literature gap will be addressed by quantifying nutrient liberation from key supraglacial habitats (snow, surface ice and near-surface ice cores) collected within the GrIS Dark Zone from the very onset of the 2017 ablation season. This will be complimented by simulations of meteorological and surface conditions derived from the regional climate model MAR, to provide a robust characterisation of the biogeochemical conditions present at the onset of glacier algal bloom development (Chapter 3).

1.3.4 Objective 3 (Chapter 4)

Quantify nutrient and organic carbon abundance in supraglacial environments of the Greenland Ice Sheet Dark Zone dominated by glacier algae, in order to explore the relationships between geochemistry and algal bloom development.

Supraglacial environments, are fundamentally oligotrophic in nature, with vital macronutrients (N and P) commonly near or below detection limits (Hodson et al., 2005; Stibal et al., 2008a; Stibal et al., 2008b; Stibal et al., 2009) and supraglacial microbial communities shown to be nutrient limited in the aqueous phase (Säwström et al., 2007; Stibal and Tranter, 2007). Yet despite this, glacier algae are able to thrive within this environment, producing blooms at such high abundances (~ 10^5 cells ml^{-1}; Nicholes et al., 2019) that they are visible from space. Whilst much is known about cryoconite-associated microbial communities and their biogeochemical cycling, the same knowledge is not currently available for glacier algae, with only three studies reporting nutrient abundance within or transecting through the Dark Zone, where glacier algae blooms dominate, yet all three sampled dispersed cryoconite as opposed to ice containing glacier algae. Quantifying nutrient and organic carbon abundance, phase and transport within surface ice environments is vital for understanding glacier algal bloom development, potential bottom-up controls on blooms within these systems and the downstream consequences of blooms on carbon and nutrient export.

A key objective of this thesis is to address this research gap by presenting the first comprehensive data set of dissolved inorganic and organic nutrients and carbon for surface ice of the GrIS Dark Zone and explore their concentrations in relation to glacier algal abundance, allowing the identification of interactions between algal biomass, nutrient abundance and phase association. This work will significantly enhance the current understanding of interactions between glacier algal blooms and key nutrient reservoirs (Chapter 4).

Chapter 2: Over winter microbial processes in a Svalbard snow pack: an experimental approach

This chapter has been published in its entirety in May, 2020 by *Frontiers in Microbiology*, with the following citation:

Holland, A. T., Bergk Pinto, B., Layton, R., Williamson, C. J., Anesio, A. M., Vogel, T. M., Larose, C., and Tranter, M., (2020). Over winter microbial processes in a Svalbard snow pack: an experimental approach, *Frontiers in Microbiology*, 11, 1029, https://doi.org/10.3389/fmicb.2020.01029.

The study was designed by me, Benôit Bergk Pinto, Tim Vogel, Catherine Larose and Martyn Tranter. Catherine Larose collected the snow and analysed the 16S qPCR samples with the assistance of Rose Layton. Rose Layton extracted the DNA, sequenced the rRNA and ran bioinformatics on the samples with the assistance of Benôit Bergk Pinto. All geochemical sample and data analysis was conducted by me. Rose Layton and Catherine Larose wrote the snow collection, DNA extraction, 16S rRNA qPCR and 16S rRNA sequencing and bioinformatics method sections. The remainder of the paper was written by me with inputs from Christopher Williamson, Catherine Larose, Rose Layton, Alex Anesio, and Martyn Tranter. All authors reviewed the final manuscript.

2.1 Abstract

Snow packs cover large expanses of Earth's land surface, making them integral components of the cryosphere in terms of past climate and atmospheric proxies, surface albedo regulators, insulators for other Arctic environments and habitats for diverse microbial communities such as algae, bacteria and fungi. Yet, most of our current understanding of snow pack environments, specifically microbial activity and community interaction, is limited to the main microbial growing season during spring ablation. At present, little is known about microbial activity and its influence on nutrient cycling during the subfreezing temperatures and 24-hour darkness of the polar winter. Here, we examined microbial dynamics in a simulated cold (-5 °C), dark snow pack to determine polar winter season microbial activity and its dependence on critical nutrients. Snow collected from Ny-Ålesund, Svalbard was incubated in the dark over a 5-week period with four different nutrient additions, including glacial mineral particles, dissolved inorganic nitrogen (DIN), dissolved inorganic phosphorus (DIP) and a combined treatment of DIN plus DIP. Data indicate a consumption of dissolved inorganic nutrients, particularly DIN, by heterotrophic communities, suggesting a potential nitrogen limitation, contradictory to phosphorus limitations found in most aquatic environments. 16S amplicon sequencing also reveal a clear difference in microbial community composition in the particulate mineral treatment compared to dissolved nutrient treatments and controls, suggesting that certain species of heterotrophs living within the snow pack are more likely to associate with particulates. Particulate phosphorus analyses indicate a potential ability of heterotrophic communities to access particulate sources of phosphorous, possibly explaining the lack of phosphorus limitation. These findings have importance for understanding microbial activity during the polar winter season and its potential influences on the abundance and bioavailability of nutrients released to surface ice and downstream environments during the ablation season.

2.2 Introduction

The cryosphere, at present, covers 10 % of the Earth's surface (Anesio et al., 2009; Lutz et al., 2017) with seasonal snow packs comprising a large portion of the frozen water landscape that encompass the cryosphere, covering over one third (~ 47×10^6 km^2) of the Earth's land surface (Hinkler et al., 2008). Snow packs play many key roles as climate regulators, such as insulating soil, permafrost and supraglacial environments from sub-freezing temperatures, as well as influencing global energy and moisture budgets (Hinkler et al., 2008). Snow packs also serve as geochemical reservoirs in Arctic environments. During winter they accumulate nutrients, then release them during the spring melt, fertilizing soils, supraglacial environments and downstream ecosystems (Kuhn, 2001; Telling et al., 2012b; Larose et al., 2013a). For example, Telling et al., (2012) found that on the Greenland Ice Sheet (GrIS) the prevalence of microbial nitrogen fixation in supraglacial environments related to the

time since snow line retreat, suggesting that the snow pack is a main source of nitrogen to these environments.

Snow is formed in the atmosphere, which leads to scavenging of nutrients and particulates, such as ammonium, nitrate aerosols, dust and bacteria, that are used in the initiation and formation of snow crystal (Jones, 2001; Kuhn, 2001; Christner et al., 2008). The content of the resulting snow pack is thus, heavily dependent on atmospheric conditions and concentrations. Snow cover undergoes physical metamorphism during the winter season, promoted by freeze-thaw cycles and temperature gradients, which further effects the distribution of solutes and nutrients within the snow pack and ice crystals themselves (Colbeck, 1991; Kuhn, 2001; Larose et al., 2010b). Recently, microbial activity has also been found to influence snow pack nutrient species, concentration, distribution and bioavailability, and even effect overlying atmospheric concentrations (Kuhn, 2001; Amoroso et al., 2010; Fujii et al., 2010; Larose et al., 2013b; Bergk Pinto et al., 2019). For example, one study has found that microbial nitrogen cycling, occurring predominantly at the base of the snow pack, leads to basal snow enriched in dissolved nitrogen compared to surface samples (Larose et al., 2013b). Yet, research on the influence of microbial activity on nutrient cycling in snow pack environments is still in its infancy.

Presently, most research on Arctic seasonal snow pack environments focuses on the polar summer, when there is 24-hour sunlight, above freezing temperatures and snow melt. This is considered to be the main growth season for microbial life residing in these environments, and an important period for nutrient export to downstream ecosystems via snow melt (Kuhn, 2001; Telling et al., 2012b; Larose et al., 2013a). In contrast, there are very few studies that investigate the polar winter, which features 24-hour darkness, sub-freezing temperatures, and extremely limited quantities of liquid water in the snow cover (Amoroso et al., 2010). As such, it is assumed that microbial life becomes inert during this season. However, this may not be the case. Several studies of ice core, glacial and sea ice and snow environments have reported active microbial communities during sub-freezing temperatures and low liquid water content (Carpenter et al., 2000; Junge et al., 2004; Miteva et al., 2007; Miteva, 2008, 2011; Maccario et al., 2019). For example, ice core studies have found certain psychrophiles, cold adapted organisms, capable of living at temperatures as low as -30 ºC (Langdahl and Ingvorsen, 1997; Price and Sowers, 2004), while Antarctic snow environments were found to contain metabolizing bacteria at -17 ºC (Carpenter et al., 2000). To date though, only one biogeochemical study of a cold Arctic snow pack environment during the polar winter has been conducted, which found that microbial oxidation of ammonium lead to the production and emission of NO, NO_2 and gaseous nitrous acid (HONO) from the snow pack at levels high enough to alter the overlying atmospheric nitrogen concentration, even in the complete absence of light and at temperatures reaching -25 °C

(Amoroso et al., 2010). It is therefore highly possible that other macronutrient cycles in Arctic snow packs are also influenced by microbial communities during the polar winter.

Dissolved inorganic phosphorus (DIP) has long been considered the ultimate limiting nutrient in glacial environments, mainly because it is principally rock-derived (Stibal et al., 2008b; Stibal et al., 2009). Most of the phosphorus (hereafter, P) found in glacial environments is sediment bound (Säwström et al., 2002; Hodson et al., 2004; Hodson et al., 2005). Sources of sediment and rock derived particles to snow pack and supraglacial environments are typically limited, comprised mostly of wind-blown debris from local terrestrial environments, atmospheric dust and melt-out of meteoric ice (Foreman et al., 2007; Wientjes et al., 2011; Bagshaw et al., 2013). Cryoconite, a material composed of nearby terrestrial rocks and dust deposited from the atmosphere that is later combined by 'glue-like' extracellular polymeric substances (EPS) secreted by cyanobacteria (Hodson et al., 2010b; Stibal et al., 2012b; Yallop et al., 2012; Musilova et al., 2016), is readily found in supraglacial environments, covering up to 10 % of glacier ablation zones in the Northern Hemisphere (Hodson et al., 2007; Hodson et al., 2010c; Anesio et al., 2009). It exists both in cryoconite holes and as dispersed material on the ice surface following the melt or wash-out of the holes (Anesio et al., 2009; Hodson et al., 2010c; Telling et al., 2012b; Yallop et al., 2012; Telling et al., 2014; Wadham et al., 2016). Cryoconite has the potential to act as a particulate inorganic phosphorus source to supraglacial environments, with potentially bioavailable phosphorus found in the particles in the order of 160 μg P g^{-1}, which is well in surplus of the maximum microbial demand of about 2 μg P g^{-1} calculated for cryoconite microbial communities (Stibal et al., 2008b). While cryoconite particles are not commonly found in snow pack environments, dust deposited from the atmosphere, that is later incorporated into cryoconite material, is commonly deposited to the snow pack via wet and dry deposition. Studies in other non-Arctic, P limited environments have found that rocks containing inclusions of P rich minerals, such as apatite and biotite, were highly colonized by local microbial communities and contained evidence of etching and bio-weathering (Rogers et al., 1998; Taunton et al., 2000a, b; Welch et al., 2002). To date, no study has investigated the ability or occurrence of Arctic microbial communities to access and utilize cryoconite particles as a source of P. We note that if microbial communities can access inorganic phosphorus from particles during the polar winter, then P may not be as limiting as previously considered and could indicate that other supraglacial microbial communities are capable of particulate inorganic phosphorus extraction too.

To this end, a laboratory experiment to examine the potential for microbial activity in a Svalbard snow pack during the polar winter was conducted. Dissolved nutrients and naturally occurring inorganic particulates were added to snow, which was then incubated in a cold (-5 °C), dark environment. The aims of this experiment were two-fold, first, to determine the dynamics of

heterotrophic bacteria in a cold, dark snow pack environment, and second, to determine if the addition of dissolved and particulate inorganic nutrients effected heterotrophic abundance and community composition. In particular, to determine if heterotrophs can access the bioavailable inorganic phosphorus in cryoconite material. We hypothesize that the addition of dissolved inorganic nutrients will stimulate heterotrophic activity in a cold, dark snow pack environment, leading to measurable changes in abundance and/or community composition. Further, we hypothesize a specialization of the heterotrophic community within the particulate inorganic nutrient addition treatment.

2.3 Methodology

2.3.1 Snow collection

Snow samples were collected during a 2012 March field campaign in Ny-Ålesund (Svalbard, Norway, 78°56'N, 11°52'E). Freshly fallen snow samples were collected into 3 L sterile sampling bags using a sterilized Teflon shovel and stored at -20 °C. Strict measures were taken to reduce human impact on sampling site and samples (Larose et al., 2009). Tyvex body suits and latex gloves were worn during sampling to avoid contamination, and gloves were worn during all subsequent handling of samples. Snow samples were maintained below 0 °C during transport to Ecole Centrale de Lyon, where they were stored at -15 °C until further utilization.

2.3.2 Microcosm set up

The snow was disaggregated in sterile Whirl-pak™ bags using a hammer in a -15 °C cold room and transferred into two large polystyrene boxes lined with sterile Whirl-pak™ bags where it was homogenized using a sterilized Teflon shovel.

Four nutrient addition experiments plus two controls were conducted, as shown in Figure 1. Nutrient additions consisted of particulate inorganic phosphorus (PIP), in the form of cryoconite particles described below, dissolved inorganic phosphate (DIP), dissolved inorganic nitrogen (DIN), and DIP plus DIN. 'Dry' controls consisted only of snow, whereas the 'wet' controls consisted of snow plus 1 mL of MilliQ water to mimic the dissolved nutrient addition (Fig. 2.1). There were 5 time points in total for the 'dry' control and the particulate phosphorus microcosms, while the 'wet' control, DIP, DIN and DIP plus DIN were only sampled at the first and last time points due to experimental constraints. Each time point had 4 replicates per treatment. The microcosms were held in 72, 2 L glass jars, which were rinsed with 10 % HCL, washed in a dishwasher using bleach and MilliQ water at 72 ºC and furnaced at 220 ºC for 20 minutes with foil covering the top for sterilization. 300 g of snow was weighed and placed in each sterilized jar. The respective nutrient spikes (see below) were

then added and homogenized. Snow and nutrients were homogenized by mixing with a sterilized spatula for roughly 10 seconds each. Controls were homogenized the same as the samples. The 'dry' control was 'homogenized' in order to prevent any discrepancy between sample and control. MilliQ water blanks were also included in three time points throughout the experiment by using MilliQ in place of snow to check for nutrient leaching from the incubation bottles. 300 mg of cryoconite particles, collected from the Greenland Ice Sheet in the summer of 2017 were added for the PIP addition. The particles were furnaced at 550 °C for 4 hours prior to addition in order to prevent any microbial or organic carbon contamination. A ratio of 2.2 mg Phosphorus per 1 g Cryoconite was assumed to determine the concentration of inorganic phosphorus added to the microcosms by the cryoconite particles (Stibal et al., 2008b). 1 ml of a concentrated solution was pipetted into the snow for the DIP, DIN and DIP plus DIN addition. DIP concentrations were chosen in order to match the concentration of inorganic phosphorus added by the cryoconite particles. A 660 ppm solution comprised of PO_4^{3-} - P was used to obtain a final concentration of 2.2 ppm after a 300-time snow dilution. DIN was comprised of ammonium (NH_4^+) and nitrate (NO_3^-), as nitrite (NO_2^-) is typically below detection limits for Arctic environments (Telling et al., 2011; Telling et al., 2012b; Wadham et al., 2016). DIN concentrations were determined from the PIP and DIP concentrations using a C:N:P ratio of 106:16:1 (Redfield et al., 1963). A 10,560 ppm solution comprised of 5,280 ppm dissolved NH_4^+ - N and 5,280 ppm dissolved NO_3^- - N was used to obtain a final concentration of 35.2 ppm after a 300-time snow dilution. 1 ml of crystal violet stain was added to an extra microcosm in order to test our method of homogenizing dissolved nutrients into the snow. The furnaced foil was replaced on top of the jars and a lid was added to keep the foil in place. The jars were stored in the dark at -5 °C until sampling.

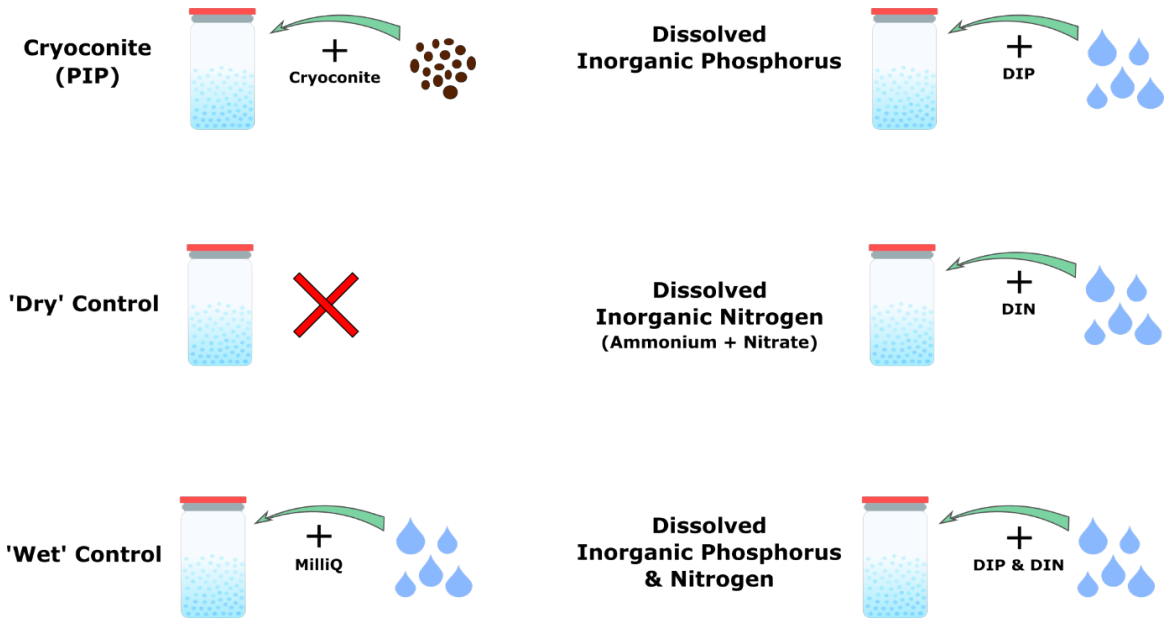

Figure 2.1: Schematic depicting the four different nutrient addition treatments and two controls.

2.3.3 Sampling

The 'dry' control and cryoconite addition microcosms were destructively sampled every five days over a total of 3 weeks, by leaving the jars to melt overnight at room temperature. The 'wet' control and dissolved nutrient addition microcosms were destructively sampled in the same way, on the initial (T0, June 26) and final (T4, July 16) time points only. pH measurements were taken of the snow meltwater using a Consort C532 meter with an epoxy gel 1M BNC electrode (Fisher Scientific) and AVS TITRINORM® pH 7 (20 °C) buffer solution (VWR Chemicals). Cryoconite treatments and 'dry' controls were filtered using a sterile plastic syringe and a 25 mm, 0.2 µm cellulose nitrate filter (Whatman™). The filtrate was collected into a pre-cleaned high-density polyethylene plastic bottle (Nalgene™; 30 mL) and stored at -20 °C, for DIP, dissolved organic phosphorus (DOP), DIN and dissolved organic nitrogen (DON) analysis. Filters retaining the cryoconite particles were stored in sterile polypropylene tubes at -20 °C prior to particulate phosphate extractions. Roughly 250 ml of meltwater from the DIP, DIN, DIN plus DIP and 'wet' control treatments were filtered onto sterile 47 mm, 0.2 µm Isopore™ membrane Millex filters using a sterile filtration unit (Nalge Nunc International Corporation). The filters were collected into sterile polypropylene tubes and immediately stored at -20 ºC prior to qPCR analysis and 16S rRNA gene Illumina sequencing. The remaining meltwater was extracted using a sterile plastic syringe and filtered through a 25 mm, 0.22 µm cellulose nitrate inline syringe filter (Whatman™) for DIP, DOP, DIN and DON analysis. The filtrate was stored in a pre-cleaned high-density polyethylene plastic bottle (Nalgene™; 30 mL) at -20 °C. Samples were maintained at these temperatures during transport and storage at the LowTex Laboratory at the University of Bristol. Dissolved nutrient samples were thawed immediately prior to analysis using a hot water bath set at a temperature of ~ 40 ºC. Procedural blanks were collected (n = 6) during the experiment, by processing deionized water in place of sample.

2.3.4 Analytical methods

2.3.4.1 Dissolved nutrients

The methods described below are similar to methods described in chapter 4 section 4.3.2, yet due to subtle differences (i.e. the use of a Gallery Plus Automated Photometric Analyzer for dissolved P analysis) and for continuity throughout the thesis, the text has been left unedited.

DIP (principally PO_4^{3-}) and total dissolved phosphorus (TDP) were determined on a Gallery Plus Automated Photometric Analyzer (Thermo Fisher Scientific, UK). TDP is the sum of DIP and DOP, and was determined by digesting the samples with a sulfuric acid persulfate digestion reagent and autoclaving at 121 ºC for 30 minutes (Jeffries et al., 1979). DOP was then calculated by subtracting DIP from TDP (i.e. DOP = TDP – DIP). The limit of detection (LoD) was 4.0 ppb (PO_4^{3-} and

TDP/DOP). LoD was determined by the mean concentration plus three times the standard deviation of procedural blanks (n = 6). Precision was ±0.43 % (PO_4^{3-}) and ±0.51 % (TDP/DOP), and accuracy was +0.12 (PO_4^{3-}) and -7.0 % (TDP/DOP), as determined from comparison with gravimetrically diluted 1000 mg L^{-1} PO_4^{3-} - P certified stock standards to a concentration of 100 ppb (Sigma TraceCERT®). All DIP and DOP concentrations were field blank corrected, using values of 6.5 ppb, n = 5 and 10.9 ppb, n = 4 respectively.

Total dissolved inorganic nitrogen (TDIN) species include NO_2^-, NO_3^- and NH_4^+, which were quantified as follows. NO_2^-, total oxidized nitrogen (TOxN) (NO_2^- + NO_3^-) and NH_4^+ were quantified spectrophotometrically using a Gallery Plus Automated Photometric Analyzer (Thermo Fisher Scientific, UK). This combination of analyses allows the original NO_3^- concentration to be determined by subtracting NO_2^- from TOxN (i.e. NO_3^- = TOxN - NO_2^-). Total dissolved nitrogen (TDN) is the sum of TDIN and DON and was determined by digesting the samples with a potassium persulfate, sodium hydroxide and boric acid reagent and autoclaving at 121 ºC for 30 minutes (Grasshoff et al., 1999). This process causes the oxidation of organic nitrogen compounds, which can then be measured as TOxN as above. DON was then calculated by subtracting the sum of the TDIN from the TDN concentration of the digested samples (i.e. DON = TDN – TDIN). LoD was determined by the mean concentration plus three times the standard deviation of procedural blanks (n = 5). The LoDs were 1.5 ppb (NO_2^-), 5.5 ppb (TOxN), 14.0 ppb (NH_4^+) and 5.5 ppb (TDN/DON). Precision was ±0.19 % (NO_2^-), ±1.1 % (NO_3^-), ±0.36 % (NH_4^+) and ±1.8 % (TDN/DON), and accuracy was +0.53 % (NO_2^-), -7.6 % (NO_3^-), -0.31 % (NH_4^+) and -11.5 % (TDN/DON), as determined from comparison with gravimetrically diluted 1000 mg L^{-1} NO_2^-- N, NO_3^- - N and NH_4^+ - N certified stock standards to a concentration of 30 ppb (NO_2^-) and 100 ppb (NO_3^-, NH_4^+, TDN/DON) (Sigma TraceCERT®). All TOxN, NH_4^+ and DON samples were field blank (n = 5) corrected by subtracting values of 8.1 ppb, 31.5 ppb and 16.8 ppb respectively. NO_2^- field blanks fell below the LoD, 1.5 ppb, n = 6, so no blank correction was applied.

2.3.4.2 Particulate phosphorus

Filters were removed from the -20 °C storage prior to extraction and left to dry in a laminar flow hood. A five-step sequential extraction scheme, adapted from Stibal et al., (2008b), was then used to operationally define phase association and bioavailability of particulate P in the cryoconite used in the incubations. The scheme used by Stibal et al., (2008b) comprises of loosely bound P (Ext. 1), iron and aluminium bound P (Ext. 2), calcium and magnesium bound P (Ext. 3), organic P (Ext. 4) and residual P (Ext. 5). Extraction 4, organic P, from Stibal et al., (2008b) scheme was combined with Extraction 5, residual P, in our scheme as the cryoconite particles had been furnaced for organic contamination prior to addition, thus there was no need to formally quantify the organic phosphorus.

Our scheme includes a step adapted from Hedley & Stewart, (1982) instead, which quantifies the phosphorus incorporated into microbial biomass in the cryoconite treatment. This step most logically fits as Extraction 3 in our scheme as it uses NaOH as the solvent, the same solvent used in Extraction 2 to determine iron and aluminium bound P. A sediment: solute ratio of ~150 mg: 3 mL used was similar to others (Hodson et al., 2004; Hawkings et al., 2016). The five-step extraction scheme used in this experiment is described in Figure 2.2. The content of P associated with different fractions of the cryoconite was quantified as DIP and DOP, as described above. Procedural blanks (n = 3) for all five extractions were conducted, using MilliQ water in place of sample to test for contamination. All DIP and DOP concentrations were blank corrected using a procedural blank. A conversion from dissolved phosphorus to particulate phosphorus concentration was applied to the cryoconite treatment and 'dry' control samples using the extract volume multiplied by the concentration divided by the sample weight.

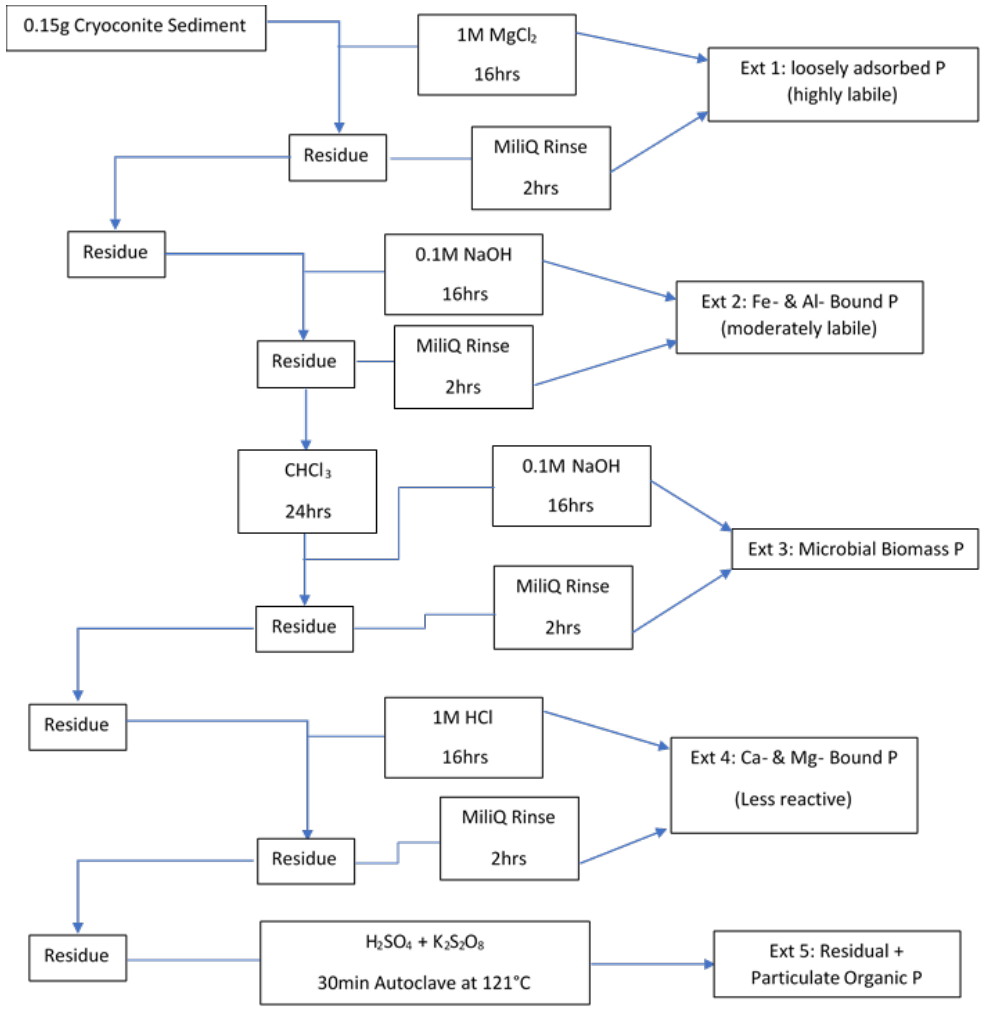

Figure 2.2: Sequential extraction method for different forms of P in the cryoconite particles added to the cryoconite treatment. All steps were done under laboratory temperature and pressure unless stated otherwise.

2.3.4.3 DNA extraction

DNA was extracted from filters of the DIP, DIN, DIN plus DIP and 'wet' control treatments using the DNeasy PowerWater Kit (Qiagen) following the manufacturer's instructions. DNA was quantified using the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Scientific).

DNA was extracted from the particles in the cryoconite addition treatment using the protocol outlined in Nicol & Prosser (2011) . Briefly, 0.5 mL CTAB phosphate buffer and 0.5 mL of phenol:chloroform:isoamyl alcohol (25:24:1) was added to each sample. Following cell lysis in a bead beater (30 s, 4 m s-1), samples were centrifuged and the upper aqueous layer retained. Residual phenol was removed by the addition of 0.5 mL of chloroform:isoamyl alcohol, followed by centrifugation and retention of the upper aqueous layer. DNA was precipitated using 30 % PEG/NaCl solution with an overnight incubation (5 degrees). The DNA pellet was collected by centrifugation, washed with 70 % ethanol and resuspended in sterile molecular grade H_2O. DNA was also extracted from untreated, furnaced cryoconite, as described above, to act as a control and test for any remaining DNA after sterilization. Two separate DNA extraction methods were utilized due to the differing material from which the DNA was extracted. DNA extracted from filters utilized a method developed for low biomass filer extractions, while a method developed for sediment extraction was better suited for the cryoconite addition treatment.

2.3.4.4 16S rRNA qPCR

Real-time qPCR analyses on the 16S rRNA genes were carried out to approximate the concentration of bacterial cells per ml of melted snow or gram of cryoconite knowing that bacteria might have more than one copy of 16S rRNA per genome. The V3 region of the 16S rRNA gene was amplified using the GoTaq qPCR Master Mix (Promega, reference A6001) using primers sequences 341F and 534R (Muyzer, 1996; Watanabe et al., 2001) on a Rotorgene 3000 machine (Qiagen). The reaction mixture of 20 µL contained 10 µL of GoTaq qPCR Master Mix, 2 µL of DNA and RNAse-free water to complete the final 20 µL volume. The qPCR 2-steps program consisted of an initial step at 95 °C for 2 min for enzyme activation, then 35 cycles of 5 sec at 95 °C and 20 sec at 60 °C hybridization and elongation. A final step was added to obtain a denaturation from 55 °C to 95 °C with increments of 1 °C.s-1. The amplicon length was around 200 bp. PCR products obtained from DNA from a pure culture of *Escherichia coli* were cloned in a plasmid (pCR™2.1-TOPO® vector, Invitrogen) and used as standard after quantification with the Broad-Range Qubit Fluorometric Quantification (Thermo Fisher Scientific).

2.3.4.5 16S rRNA sequencing and bioinformatics

Microbial community structure was determined by MiSeq Illumina amplicon sequencing of the bacterial V3-V4 region of the 16S rRNA gene using the Library Preparation Workflow recommended by Illumina (Illumina, Inc., San Diego, CA, United States). The V3-V4 region of the 16S rRNA gene was amplified using the Platinum Taq Polymerase (ThermoFisher Scientific) using the primer set 783F – 1046R from (Klindworth et al., 2013) resulting in the following sequences:5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' as the forward primer sequence, and 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3' as the reverse primer sequence (Illumina, Inc., San Diego, CA, United States). The PCR program used was: 95 °C for 3 minutes, 35 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds and 72 °C for 30 seconds, then a final step of 72 °C for 5 minutes. Paired end sequencing was then carried out on a MiSeq sequencer (Illumina) at the laboratory in Lyon. Sequencing primers were removed using CutAdapt and filtered, trimmed and processed using Dada2. Taxonomy was assigned to the inferred sequence variants using the Dada2 formatted RDP dataset (RDP trainset 16).

2.3.5 Data analysis

All geochemical measurements below the LoD were considered to be 0 for all statistical analyses. All DIN, DON, DIP and DOP data were water blank-corrected using values from the respective procedural blanks. All PIP and POP data were water blank-corrected using values from the respective procedural blanks. Additionally, all blank corrected values that were negative were assumed to be 0 for all statistical analyses. Dada2 results and annotations were imported into R (R Development Core Team, 2011) and analyzed with the R package 'phyloseq' (McMurdie and Holmes, 2013). Amplicon sequence variants (ASV) not taxonomically assigned to Bacteria were excluded from further analysis. Samples were rarefied to equal the sample with the lowest read counts using the 'rarefy even depth' phyloseq function prior to Alpha diversity calculations with the 'estimate richness' function. ASV count matrices were normalized by relative abundance prior to statistical analyses. Statistical analyses were performed in RStudio v.1.1.414 (RStudio, Inc 2018). Identification of statistical differences between nutrient concentrations, bacterial abundance of dissolved nutrient treatments, sample date and treatment type were achieved using 2-way and 1-way analysis of variance (ANOVA) comparisons, with post-hoc Tukey HSD analysis applied to all significant ANOVA results. T-tests were used to compare observed Alpha diversity measurements for each treatment between time points. Homogeneity of variance and normality of distribution were tested prior to all parametric analyses, and model assumptions were verified by examination of model criticism plots.

2.4 Results

2.4.1 Dissolved inorganic nutrients

DIP and TDIN decreased significantly over the 5-week sampling period. The decrease in DIP and TDIN concentrations was not homogenous across all treatments, however. Both DIP and TDIN concentrations decreased significantly in the combined DIN plus DIP treatment only ($F_{1,10}$=17.5, $p<0.01$ and $F_{1,10}$=1.0, $p<0.01$, respectively (Fig. 2.3A & B). DIP concentrations in the combined treatment decreased from 2.02 ± 0.05 ppm to 1.57 ± 0.09 ppm while TDIN concentrations decreased from 33.8 ± 0.92 ppm to 25.7 ± 1.9 ppm. TDIN is composed of NH_4^+ and NO_3^-, as previously described, therefore, changes in each species of nitrogen were investigated in order to determine which N species was in higher demand. NH_4^+ decreased significantly from 16.6 ± 0.39 ppm to 12.4 ± 0.87 ppm in the combined treatment only ($F_{1,10}$=1.6, $p<0.05$) (Fig. 2.3C). NO_3^- was the only added dissolved nutrient that significantly decreased in both the single and combined treatments ($F_{1,10}$=0.1, $p<0.05$, for both) (Fig. 2.4). NO_3^- concentrations decreased from 13.8 ± 0.42 ppm to 10.3 ± 1 ppm in the DIN addition treatment and from 17.2 ± 0.52 ppm to 13.2 ± 1 ppm in the combined treatment. No significant change occurred in any dissolved nutrient concentration in the 'wet' control, nor in DIP concentrations in the DIP treatment.

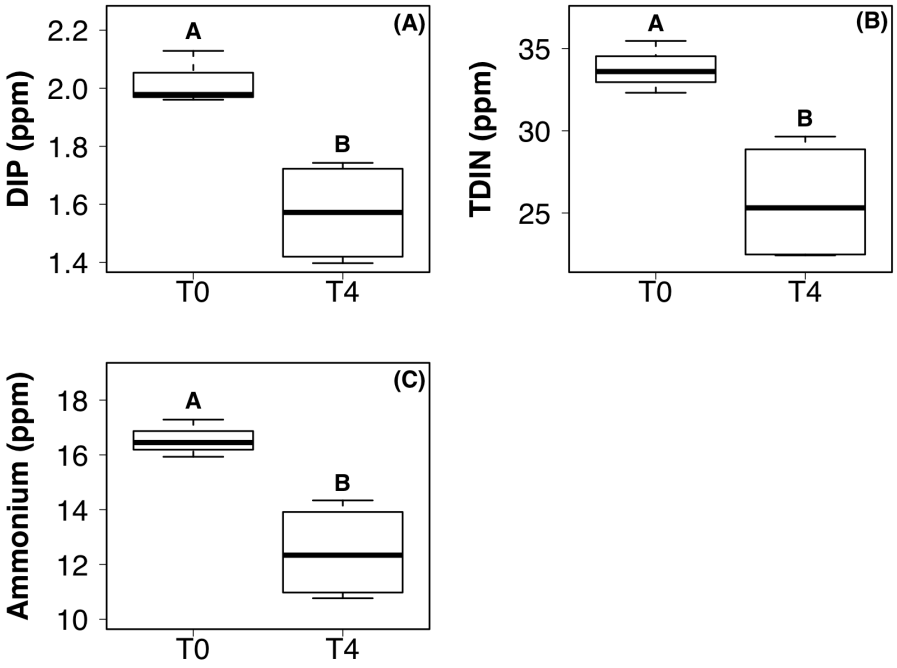

Figure 2.3: Boxplots showing the median, upper and lower interquartile range of dissolved inorganic phosphorus (DIP) **(A)**, total dissolved inorganic nitrogen (TDIN) **(B)** and ammonium **(C)** concentrations for the combined DIP and dissolved inorganic nitrogen (DIN) treatment at the initial (T0) and final (T4) time points. *Uppercase letters* denote homogeneous subsets derived from post hoc Tukey HSD analysis on a significant two-way ANOVA in relation to the time point and treatment.

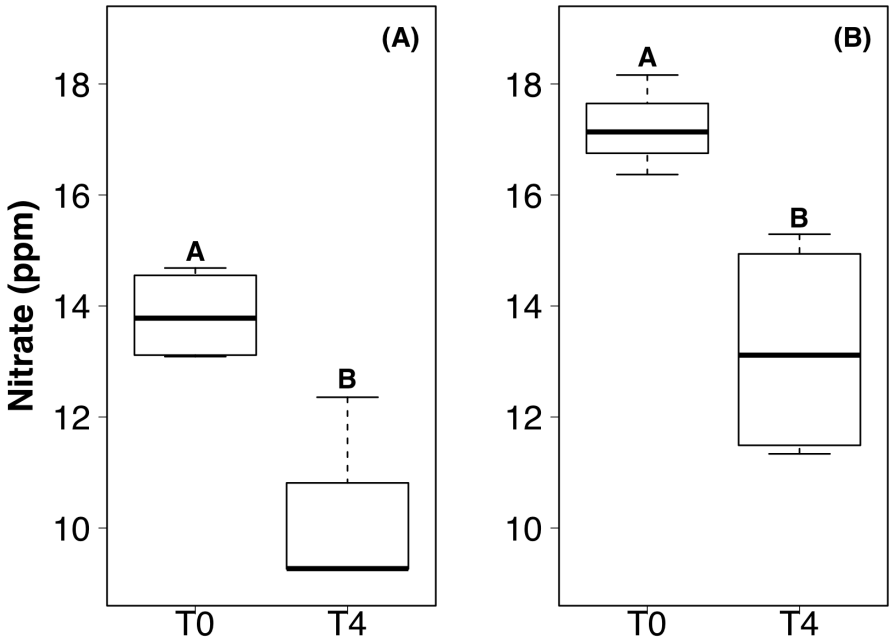

Figure 2.4: Boxplots showing the median, upper and lower interquartile range of nitrate concentrations for dissolved inorganic nitrogen (DIN) treatment **(A)** and combined dissolved inorganic phosphorus (DIP) and DIN treatment **(B)** at the initial (T0) and final (T4) time points. *Uppercase letters* denote homogeneous subsets derived from post hoc Tukey HSD analysis on a significant two-way ANOVA in relation to the time point and treatment.

2.4.2 Bacterial abundance and taxonomy

The 'wet' control bacterial abundance was the only treatment that exhibited a significant decrease in abundance between the initial and final time points ($F_{3,20}$=6.27, P<0.05) (Fig. 2.5A). Bacterial abundance in all dissolved nutrient addition treatments were elevated in the final time point compared to the initial time point (Fig. 2.5D, G & J). Bacterial abundance in the cryoconite treatment displayed an overall increasing trend across the timeseries, with all time points having at least a two order of magnitude increase compared to the untreated, furnaced cryoconite control and the final time point (T4) containing an order of magnitude higher bacterial abundance compared to all other time points (Fig. 2.5M).

441 genera from 25 phyla were detected across the 4 treatments and the 'wet' control. Betaproteobacteria, more specifically *Massilia*, dominated the dissolved nutrient addition treatments and 'wet' control, whereas Alphaproteobacteria, more specifically *Methylobacterium*, had the highest abundance in the cryoconite treatment (Fig. 2.6). There was also a clear diversification of the microbial community present in the cryoconite treatment compared to the untreated, furnaced control cryoconite particles, as only two common genera were detected, *Chryseobacterium* and

Propionibacterium (Figs. 2.5O & 6). Alpha diversity measurements revealed an overall decrease in genera richness between T0 and T4 for the 'wet' control and DIN treatment, as seen in Figure 2.5B, C, E & F. Alpha diversity increased over the time series for both the combined treatment (Fig. 2.5K) and DIP treatment, with a significant increase found in the DIP treatment ($t = -2.82$, $p<0.05$) (Fig. 2.5H). Alpha diversity also increased significantly after T0 in the cryoconite treatment ($t = -3.23$, $p<0.05$) (Fig. 2.5N).

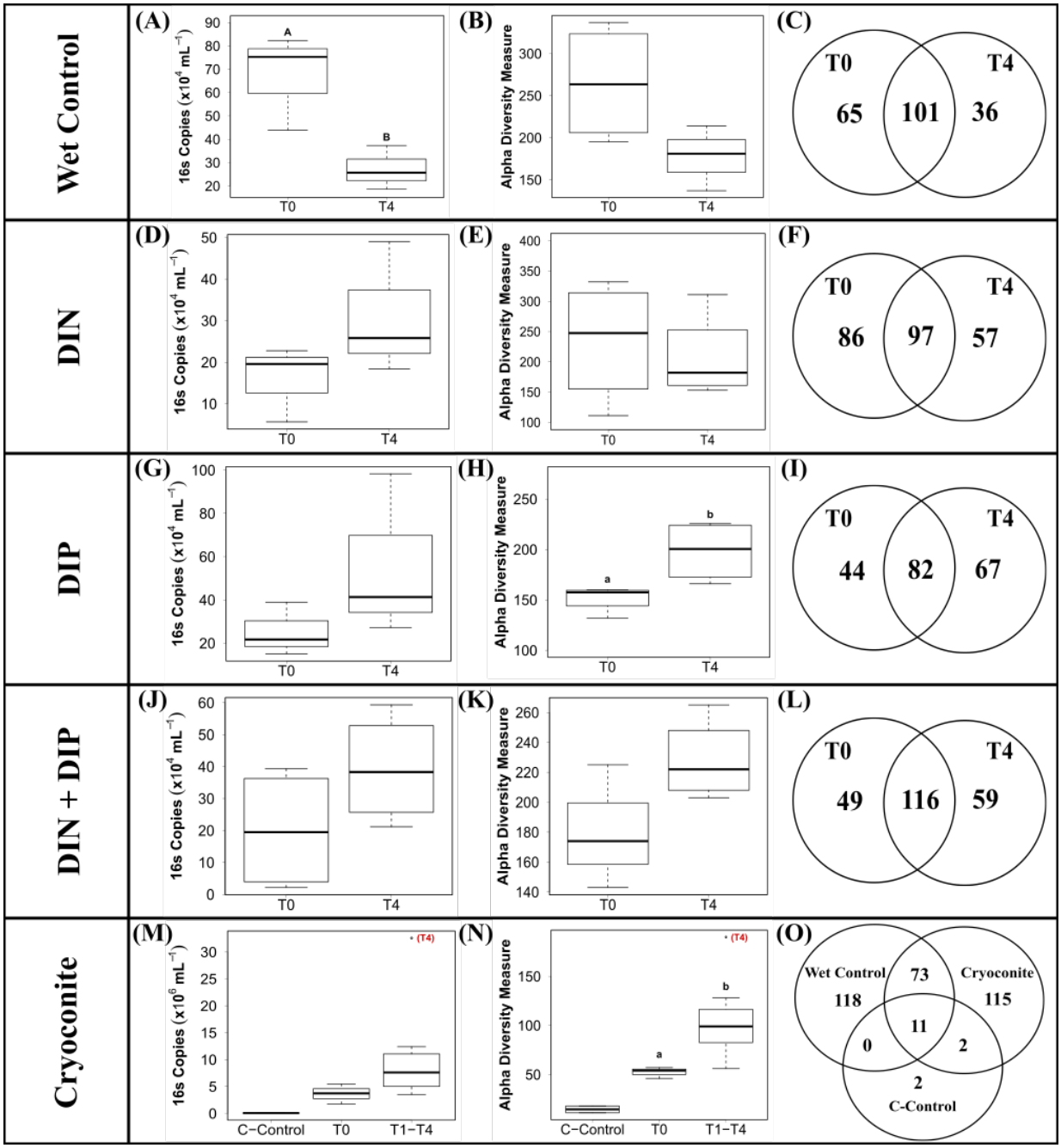

Figure 2.5: Boxplots showing the median, upper and lower interquartile range of bacterial abundance **(A,D,G,J,M)** and observed Alpha diversity measurement **(B,E,H,K,N)** and Venn diagrams depicting genera richness **(C,F,I,L,O)** for all treatments. T0 and T4 represent initial and final time point, respectively. C-Control in the cryoconite treatment represents untreated, furnaced cryoconite control. DIN and DIP represent dissolved inorganic nitrogen and dissolved inorganic phosphorus, respectively. *Uppercase letters* denote homogeneous subsets derived from post hoc Tukey HSD analysis on a significant two-way ANOVA in relation to treatment and time point. *Lowercase letters* denote t test comparisons in relation to time point.

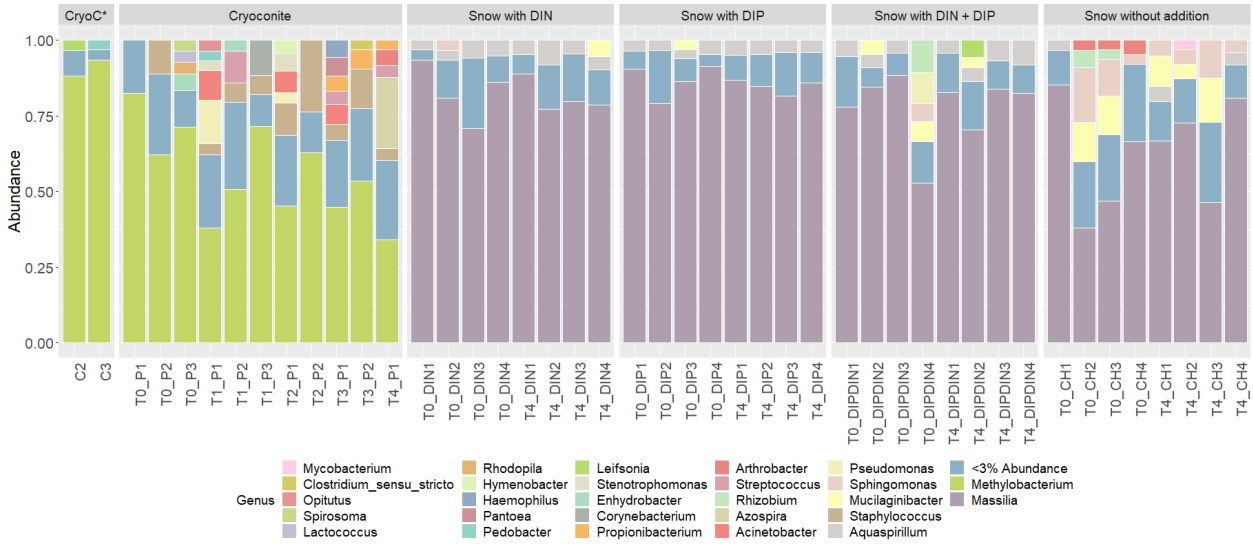

Figure 2.6: Relative abundances of taxa representing > 5 % abundance and classified to genus level across all treatments and time points. CryoC* represents untreated, furnaced cryoconite control, with C2 and C3 representing the two replicates. T0 – T4 represent time points 0 – 4, respectively. DIN and DIP represent dissolved inorganic nitrogen and dissolved inorganic phosphorus, respectively. P/DIN/DIP/DIPDIN/CH 1 – 4 represent the four replicates for each time point per treatment.

2.4.3 Cryoconite particulate phosphorus

The mean total PIP content in the cryoconite particles added to the microcosms was 649.7 ± 46.8 µg g^{-1}, which falls within the range of P content within most rock types in the Earth's crust (230 – 670 µg P g^{-1}) (Hodson et al., 2004). Most PIP (82.8 ± 0.74 %) was present in Extract 2 ('Fe- and Al-bound'), followed by Extract 4 ('Ca- and Mg-bound'; 8.8 ± 0.52 %), Extract 5 ('Residual + Organic P'; 4.4 ± 0.11 %), Extract 3 ('Microbial Biomass'; 4.3 ± 0.35 %) and Extract 1 ('Loosely adsorbed P'; 0.51 ± 0.23 %). One-way ANOVAs revealed significant changes in PIP content in Extracts 1-3 (Ext 1: $F_{4,15}$=30.9, p<0.0001, Ext 2: $F_{4,15}$=6.8, p<0.001, Ext 3: $F_{4,15}$=3.5, p<0.01). A spike in PIP content found in Extracts 1-3 occurring on July 6th, time point 2 (T2), midway through the time series, drives this trend. DIP concentrations quantified from the filtrate of the cryoconite treatment revealed a significant decrease in concentration on the same date ($F_{4,15}$=84.3, p<0.0001). This relationship can be seen in Figure 2.7, which depicts the percent changes for Extracts 1-3 and DIP ($F_{4,15}$=27.2, p<0.0001, $F_{4,15}$=3.1, p<0.01, $F_{4,15}$=3.1, p<0.01 and $F_{4,15}$=58.6, p<0.0001, respectively). DOP and POP results from the filtrate and extracts displayed no change over time, apart from Extract 3. Extract 3, which quantified the organic phosphorus within the microbial biomass in the cryoconite treatment, demonstrated an initial decrease, followed by a sudden increase after T2 (Fig. 2.8).

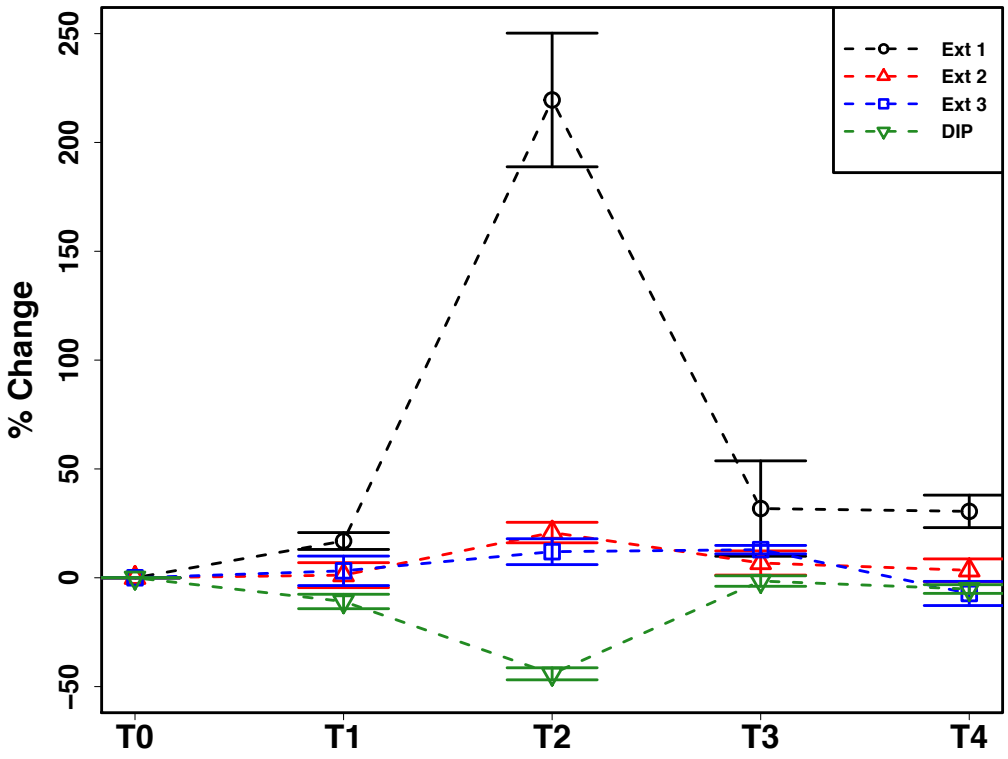

Figure 2.7: Mean ± SE of the percent change in particulate phosphorus content of cryoconite particles (Ext 1-3) and dissolved inorganic phosphorus (DIP) concentration of filtrate from cryoconite treatment throughout the time series, $n = 4$ for each time point of each extraction.

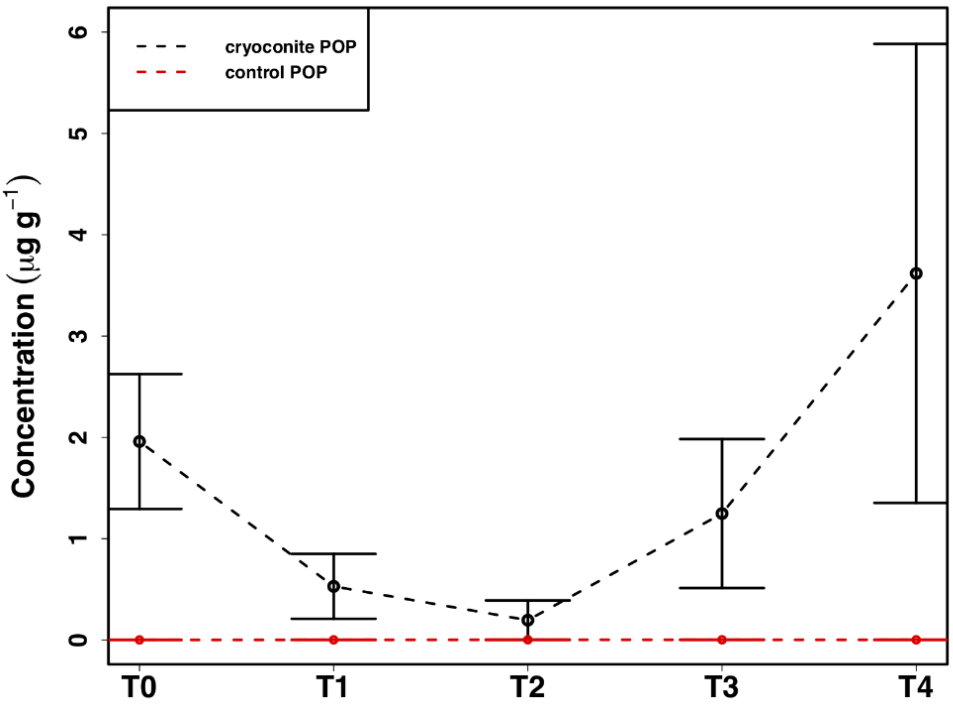

Figure 2.8: Mean ± SE of the particulate organic phosphorus (POP) content of heterotrophic biomass associated with cryoconite particles in the cryoconite treatment throughout the time series and the 'dry' control, $n = 4$ for each time point of each treatment.

2.5 Discussion

In this present study we show evidence of microbial community dynamics and their effect on nutrient cycling within a simulated cold, dark snow pack environment. This has implications for how we view microbial activity during the polar winter, a season that has previously been considered inert. As the 24-hours of darkness and subfreezing temperatures of the polar winter can comprise up to 6 months of the year, it is important to understand microbial influences on nutrient cycling as it could affect nutrients released during the ablation season.

2.5.1 Varied heterotrophic community response to dissolved nutrient additions

We demonstrate here the potential for active microbially-mediated nitrogen cycling in snow-packs, a dynamic already known to occur during spring and summer (Larose et al., 2013b), under simulated polar winter conditions. A clear community response was detected upon the addition of DIN, with an elevated final abundance and subsequent decline in genera richness suggesting potential specialization of heterotrophic communities, with genera better adapted to a nitrogen enriched environment potentially outcompeting other less suited bacteria (Fig. 2.5D, E & F). This was supported by the presence throughout the time series of bacteria associated with the nitrogen cycle, e.g. *Bacillus* and *Caulobacter* genera known to perform N fixation (Różycki et al., 1999) and as efficient NH_4^+ scavengers (Poindexter, 1981), respectively, and the emergence of others, e.g. *Nitrospira* known for the oxidation of nitrite to nitrate (Spieck and Bock, 2015), by the final time point. As high, irregular inputs of nitrogen are common during the polar winter, via wet and dry deposition (Björkman et al., 2014), specialization in associated heterotrophic communities is likely to occur *in-situ*, with consequences for the nitrogen cycle. For example, changes in bacterial assemblage composition associated with DIN addition during the present study likely underlaid the observed preferential consumption of NO_3^- over NH_4^+, thus driving variability in the relative abundance of nitrogen species through time (Fig. 2.4A), consistent with spring and summer *in-situ* observations (Larose et al., 2013b).

In contrast to the assertion of DIP as the key limiting nutrient in glacial environments (Hodson et al., 2004; Stibal et al., 2008b; Stibal et al., 2009), DIP was only measurably consumed in the combined dissolved nutrient treatment during the present study; for which we propose two possible explanations. Firstly, it is likely that the abundance of DIN influenced the efficiency of DIP consumption, as evidenced by DIP being measurably consumed in the combined treatment only. With the absence of DIN in the DIP treatment, reduced DIP consumption would be required to maintain stoichiometric homeostasis (e.g. a C:N:P ratio of 20:4:1 for freshwater heterotrophic bacteria; (Fagerbakke et al., 1996; Vrede, 1998), whereas in the combined treatment, both DIN and DIP were consumed effectively. Second, DIP may not be as limiting in supraglacial environments as

previously believed (Hodson et al., 2004; Stibal et al., 2008b; Stibal et al., 2009), considering that DIN was consistently consumed during the present study when concentrations were artificially elevated. Such trends have also been reported in recent studies concerning both snow pack and surface ice environments, which suggested DIN to be in higher demand than DIP (Larose et al., 2013b; Holland et al., 2019). Overall, there is a clear response by the microbial community to all nutrient additions over the course of just 5-weeks in a cold, dark snow pack, indicating that the 24-hour darkness and subfreezing temperatures of the polar winter may not be as limiting as previously thought.

2.5.2 Potential extraction of particulate phosphorus

In addition to the utilization of dissolved nutrient resources, we further demonstrate the potential for microbial extraction of particulate phosphorus from cryoconite particles in snow pack environments in the present study. Inverse relationships observed between DIP and PIP pools suggested the capacity for phase changes between dissolved and loosely bound phosphorous fractions. For example, a simultaneous increase in PIP and decrease in DIP concentrations at T2 (Fig. 2.7) indicated rebinding of DIP to available receptor sites on cryoconite particles (most likely as Extract 1), producing new, loosely bound PIP accessible to microbial communities via different methods such as carbonation weathering, dissolution or acidification through the production of organic acids (Welch et al., 2002), as described below. DIP adsorbed onto particles, whether as loosely bound P or on poorly ordered Fe- and Al- oxyhydroxides, has been found to be readily bioavailable in cryospheric environments (Hodson et al., 2004; Stibal et al., 2008b). Extract 1, the most labile and readily bioavailable form of PIP (Stibal et al., 2008b), accounted for the smallest portion of total PIP extracted during the present study, suggesting consumption by microbial communities. Results from the present study are analogous to those in sediment rich, oligotrophic aquatic environments, whereby P adsorption to particulates has a significant impact on the fate and distribution of bioavailable P in the environment (Müller et al., 2006). An initial decrease in P within the microbial biomass, followed by an increase after T2, suggests a possible utilization of internal phosphorus stores, perhaps through storage in polyphosphates, before being able to access the newly adsorbed, bioavailable P (Fig. 2.8). This storage and utilization of polyphosphate molecules as an energy source has been well documented in many other heterotrophic communities (Kornberg, 1995; White et al., 2010; Achbergerová and Nahálka, 2011; Tocheva et al., 2013).

P liberation from particulate sources has previously been documented by microbes via etching, production of organic ligands, influencing the solution saturation state or most commonly lowering the pH either at the mineral surface or the bulk pH through the production of organic acids (Welch et al., 2002). The pH of the cryoconite treatment in the present study varied only slightly with an

average pH of ~ 6.02 ± 0.13. Even though our pH was slightly acidic, production of organic acids typically lower the bulk pH to between 3.5 - 5 (Welch et al., 2002), if microbial acidification was used to mine the loosely adsorbed P, then the pH may have only been altered at the particle surface, enough to access the labile P, but not enough to effect the bulk pH, or they utilized an alternate method such as the production of pyruvate, which has also been found to aid the dissolution of P containing minerals without altering pH (Welch et al., 2002).

2.5.3 Increased abundance and diversity overtime in particulate associated heterotrophic community

We demonstrate a potential colonization of the cryoconite particles over time by genera found in the snow pack environment, as suggested by a combined increase in bacterial abundance and diversity over time in the cryoconite treatment and compared to the untreated, furnaced cryoconite control (Fig. 2.5M & N). A clear diversification in the main genera found in the cryoconite treatment compared to the 'wet' control and untreated, furnaced cryoconite is also present (Fig. 2.6), indicating that certain genera from the snow pack environment might be better suited for colonizing the particles. Only 73 shared genera were detected between the treated cryoconite and 'wet' control, representing the genera that could act as early particulate colonizers (Fig. 2.5O). For example, *Massilia* is found in both the treated cryoconite and 'wet' control. *Massilia* is not only a Betaproteobacteria, a class which has been previously identified as early colonizing bacteria with high abundance in snow pack environments that may play a key role in mineral weathering in debris covered glaciers (Pianka, 1970; Fierer et al., 2007; Fierer et al., 2010; Larose et al., 2010; Zumsteg et al., 2012; Hell et al., 2013; Franzetti et al., 2013), but also a genera that has species linked to solubilizing phosphate (Zheng et al., 2017). In fact, there is a clear increase in overall Betaproteobacteria abundance in the final time point of the cryoconite addition treatment compared to previous time points (Fig. 2.6). Other non-betaproteobacteria such as *Arthrobacter*, that have been shown to assist in mineral dissolution by lowering pH, are also found among the 73 potential colonizers (Barker et al., 1998; Welch et al., 2002). Based on the notable increase in bacterial abundance and Alpha diversity after T0 (Fig. 2.5M & N), it seems likely that given more time the colonization, and potential utilization, of the particles by microbes would have continued to increase. Any potential colonization or extraction of particulate inorganic substrates by heterotrophic communities in snow pack environments is likely to occur in other supraglacial or Arctic environments as snow packs have been considered to seed microbial communities in underlying environments during the ablation season (Musilova et al., 2015).

2.6 Conclusion

Our study indicates the presence of an active heterotrophic community in a cold (-5 ºC), dark snow pack environment. Snow collected from a seasonal snow pack in Ny-Ålesund, Svalbard was

incubated with dissolved and particulate nutrient additions to determine whether the additions, in particular the naturally occurring inorganic substrate, affected heterotrophic abundance or community composition. Our results suggest a specialization within the heterotrophic community of genera known to influence N cycling in the presence of excess DIN, but diversification when abundant DIP is present. As high inputs of N are common to snow pack environments during the polar winter, this specialization may occur *in-situ*, leading to efficient N cycling by heterotrophic bacteria. We also find strong evidence for the utilization of particulate phosphorus sources from an inorganic glacial sediment by the heterotrophic community, which also exhibits colonization by certain genera from the snow pack environment, e.g. *Betaproteobacteria*, known to be early colonizers of particulates. The ability of heterotrophic bacteria to access particulate phosphorus in snow pack and potentially other supraglacial environments may thus be a key factor influencing survival in oligotrophic glacial environments. Overall, we demonstrate that changes in heterotrophic community abundance and composition in snow pack environments influence nutrient cycling during the polar winter, which in turn impacts the speciation, abundance and bioavailability of nutrient resources relative to their depositional state, and thus their roles in supraglacial and downstream ecosystems during the ablation season.

Chapter 3: Dissolved N speciation and concentrations during snowmelt in the Dark Zone of the Greenland Ice Sheet: evidence for microbial activity in the snow pack and underlying ice during the spring thaw.

This chapter has been prepared as a research article for future submission to *Biogeochemistry*, with the following reference:

> **Holland A. T.**, Williamson, C. J., Tedstone, A. J., Anesio, A. M. and Tranter, M. (in preparation). Dissolved N speciation and concentrations during snowmelt in the Dark Zone of the Greenland Ice Sheet: evidence for microbial activity in the snow pack and underlying ice during the spring thaw, *Biogeochemistry*.

This study was conceived and designed by me with assistance from Christopher Williamson and Martyn Tranter. All sample and data analysis were conducted by me. Andrew Tedstone provided data from MAR and wrote the snowmelt modelling method section. The remaining manuscript was written by me with input from Martyn Tranter, Christopher Williamson and Alex Anesio.

3.1 Abstract

This study provides a preliminary dataset on the concentration and speciation of dissolved nitrogen (N) in snow, meltwater and ice within the Dark Zone of the Greenland Ice Sheet. The ripe, partially leached snow pack near S6 on the K-Transect in south-west Greenland gave rise to meltwaters with relatively constant nitrate (NO_3^-) concentrations, approximating the snow pack average (1.1 μM). By contrast, ammonium (NH_4^+) (0 – ~ 4 μM) and dissolved organic nitrogen (DON) (0 – ~ 3 μM) concentrations were more variable, while their average snow pack concentrations were 1.0 and 0.4 μM, respectively. This variability could be the result of microbial uptake and production within the melting snow pack. Experimental snow pits flooded, due to rolling topography of the ice surface, exposing pooled meltwater at the snow-ice interface that appeared to scavenge DON (~ 1 – 8 μM) and possibly NO_3^- (~ 1 – 2 μM) from the underlying ice, whose initial surface may have been a continuum of superimposed ice and weathering crust from the previous season. The shallow meteoric ice beneath the snow pack had high concentrations of DON and NH_4^+, 6.5 and 2.6 μM, respectively, while NO_3^- concentrations were similar to the snow pack (1.1 μM). The absence of NH_4^+ in the snowmelt traversing the snow-ice interface also points to microbial activity occurring at this boundary layer. Calculated temperature from MAR, a regional melt model, predicts that at least 10 cm of the surface ice below the snow pack is at 0 °C, likely caused by penetration of solar radiation through the shallow snow pack. As solar radiation is transmitted through the snow pack and onto the ice surface it is absorbed by dark particulates (or light adsorbing impurities), including glacier algae, which likely results in localized melting and production of micro-environments in which the glacier algae can metabolise before the surface snow cover disappears. Hence, glacier algal blooms may commence before the snow pack has completely melted and occur at a time when meltwaters percolating into the remains of the weathering crust from the previous melt season are enhanced in nutrients, as a result of scavenging from the snow pack, superimposed ice layers and the former weathering crust itself.

3.2 Introduction

The 'Dark Zone' on the south-west margin of the Greenland Ice Sheet (GrIS) is an annually reoccurring area of surface ice, ~ 50 km wide and some 20 – 30 km from the ice margin, with albedo measurements approaching as low as 0.2 (Wientjes and Oerlemans, 2010; Box et al., 2012; Stroeve et al., 2013; Cook et al., 2020). This site has captured the attention of scientists in recent years as its increasing area and duration over the past 2 decades has coincided with increasing surface melt (Rignot and Kanagaratnam, 2006; Rignot et al., 2011; Shepherd et al., 2012). The factors which control the extent and duration of the surface darkening have been contested, and include high concentrations of Holocene dust melting out of meteoric ice (Wientjes and Oerlemans, 2010; Wientjes et al., 2011; Wientjes et al., 2012), increased atmospheric deposition of black or brown carbon

(Keegan et al., 2014; Tedstone et al., 2017; Ryan et al., 2018) and algal blooms (Yallop et al., 2012; Williamson et al., 2019; Williamson et al., 2020). Interest in the association between algal blooms and surface darkening has grown recently, with studies now confirming that Streptophyte glacier algae, which contain a unique dark UV/VIS absorbing pigment, purpurogallin phenolic pigment (Remias et al., 2012b; Yallop et al., 2012; Stibal et al., 2017a; Cook et al., 2020; Williamson et al., 2020), are a significant factor responsible for the interannual variability in the extent, duration and scale of the Dark Zone (Tedstone et al., 2017; Tedstone et al., 2020).

The factors which stimulate algal blooms are presently being investigated (Stibal et al., 2015; Williamson et al., 2018), with the availability of nutrients likely a key factor, yet, the specifics of this assertion are limited by the low number of pertinent studies to date (Telling et al., 2012b; Stibal et al., 2015; Wadham et al., 2016; Holland et al., 2019; McCutcheon et al., Submitted). We contend that a knowledge of nutrient inputs from melting snow to ice surface environments is essential for understanding the development of surface microbial communities during the early stages of the melt season, as snowmelt has already been shown to be a key nutrient source to many Arctic (Telling et al., 2011; Telling et al., 2012b), Antarctic (Telling et al., 2014), and other high altitude habitats (Bales et al., 1990; Williams et al., 1991) during the early ablation season. Concentrations of nutrients in snowmelt are not typically constant, particularly during the 'ionic pulse' of the early melt period. The ionic pulse occurs when solutes fractionate into snowmelt at higher concentrations than are present in the overall parent snow cover (Davies et al., 1982; Hewitt et al., 1991; Harrington and Bales, 1998), with the initial 30 % of meltwater often containing ~ 50 – 80 % of the total snow pack solute load (Cragin et al., 1996; Johannessen et al., 1975; Johannessen and Henriksen, 1978). The elution is mostly caused by solutes being excluded from the ice crystal lattice during metamorphism. A secondary effect, called preferential elution, is the result of the different solubilities of individual solute species in ice (Cragin et al., 1996), causing insoluble ions to elute earlier and in higher concentrations than soluble ones. Several factors can accentuate the ionic pulse, such as freeze-thaw cycles, bands of solute-rich snow or ice and deeper snow cover (Bales et al., 1990; Tsiouris et al., 1985; Cragin et al., 1996). Banding of solute is routinely caused during melt-freeze cycles, when surface melt refreezes at depth in the snow pack (Brimblecombe et al., 1987; Harrington and Bales, 1998). In particular, the meltwater which refreezes at the base of the snow pack above the cold ice, referred to as superimposed ice, is often rich in solutes (Bales et al., 1990).

Snowmelt may be the main nitrogen (hereafter referred to as N) input in some supraglacial environments, comprising > 95 % of the total N input on a Svalbard glacier for example (Hodson et al., 2005). Recent average annual nitrate (NO_3^-) concentrations in snowfall on Greenland are of the order of 2.8 – 5.6 μM, as recorded at Summit Station (Fibiger et al., 2016). Concentrations may be

lower nearer to the margin, with one study reporting values of 1.0 ± 0.3 μM near the south-west margin of the GrIS on the K-Transect (Telling et al., 2012b). Ammonium (NH_4^+) concentrations in snow are usually lower, with those from Summit ranging from 0.35 – 0.89 μM for 1991 – 1994 (Silvente and Legrand, 1993; Dibb et al., 1998), whereas values were below detection on the K-Transect (Telling et al., 2012b). At present, there is little available data on dissolved organic nitrogen (DON) concentrations in snow pack environments.

Ablating surface ice can also be an important N source later in the season after the snow cover has melted, particularly when N concentrations in snow and ice are comparable. This is the case in the three studies of the Dark Zone bare ice habitats to date, where dissolved inorganic nitrogen (DIN) (comprising mostly of NO_3^- and NH_4^+) concentrations ranged from 0.6 to 1.3 μM (Telling et al., 2012b; Holland et al., 2019; Wadham et al., 2016). These values are similar to those reported in a review of Greenland ice core N concentrations, which had a mean DIN concentration of 1.4 μM (Wolff, 2013). NO_3^- concentrations typically exceed those of NH_4^+ in meteoric ice beneath surface ice, with average values of 0.97 and 0.45 μM, respectively (Fuhrer et al., 1996; Mayewski et al., 1994; Wolff, 2013). Yet, NO_3^- concentrations have been found to decrease in surface ice in the presence of microbes, reportedly dropping to zero (Telling et al., 2012b; Holland et al., 2019), whereas NH_4^+ concentrations were found to increase, reaching values of up to 1 μM (Holland et al., 2019). Surface ice concentrations of DIN are also significant in that they were found to exceed the estimated nitrogen requirement for balanced microbial growth in cryoconite holes located 2 – 7.5 km inland from the margin of the GrIS during the late ablation season (Telling et al., 2012b). Yet, the persistent presence of N fixation in these habitats suggest that other supraglacial microorganisms, such as glacier algae (Yallop et al., 2012; Williamson et al., 2019), were also utilizing N released from ablating surface ice (Telling et al., 2012b), hence, the ablating surface ice likely acts as a significant N source for several supraglacial habitats and microbial communities. There are relatively few reported values of DON for surface ice in the literature at present, with one study reporting relatively high DON concentrations (14.0 μM) in surface ice colonized by glacier algae in the GrIS Dark Zone (Holland et al., 2019). Average DON concentrations of 1.6 μM were also reported for surface ice containing dispersed cryoconite in the same location (Wadham et al., 2016), whereas quantifiable concentrations of DON could not be found in the surface ice closer to the GrIS glacier margin (Telling et al., 2012b).

Knowledge of the controlling factors on the initiation of glacier algal blooms is currently limited. Recent work has shown that glacier algae, which colonise ice environments containing water pockets down to ~ 30 cm below the ice surface, are frozen into the ice during the end of the melt season and remain viable overwinter beneath the snow cover (Nicholes et al., 2019). During spring, when the

snow pack thins, the algal cells become activated by light penetrating the snow cover (Fig. 3.1), potentially allowing them to absorb the solar radiation, due to their dark pigmentation, and melt the surrounding ice, forming a niche in which to first metabolise. These niches are then eventually bathed by early ablation season snow and surface ice melt that soaks through the base of the snow cover and into the rotting underlying ice. Hence, a knowledge of the composition of early snow and ice melt that wet the ice surface in spring may be valuable as a potential factor controlling of the initiation of glacier algal blooms in spring.

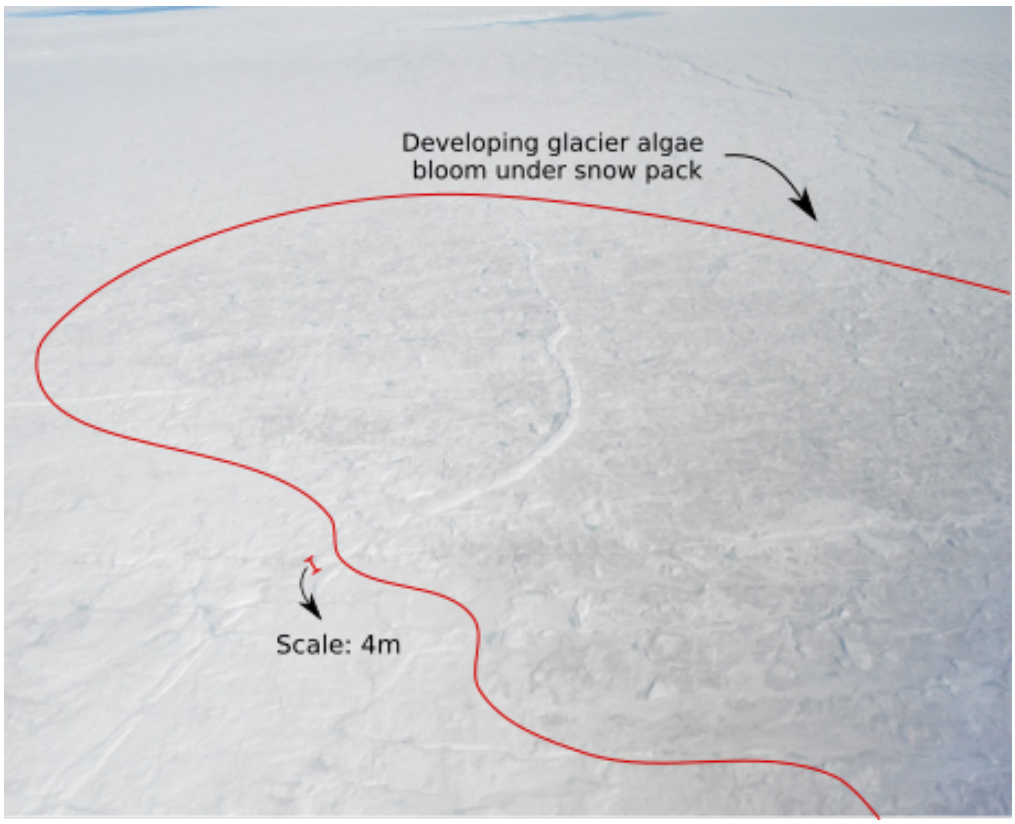

Figure 3.1: The onset of glacier algal blooms near the snowline in the vicinity of the K-Transect, SW Greenland (see Fig. 3.2A). The snow-filled supraglacial channel running centre image through the bottom left has a width of ~ 4 m for scale. It is evident that glacier algae (dark areas, centre right) are darkening the surface even as the last snow is ablating.

The aims of the present study, therefore, are twofold. First, to document DIN and DON concentrations in the snow pack, surface ice and snowmelt during the ablation season near to S6 on the K-Transect within the GrIS Dark Zone. Second, to infer the key processes which regulate N species and concentrations at this key phase of the life cycle of glacier algae, when the first photosynthetic activity of the year is about to occur. Our goal is to provide a preliminary dataset of N concentration, phase and species association released from snow and ice melt in the GrIS Dark Zone

at the onset of melt, which will inform future laboratory incubation experiments of the likely speciation and range of N concentrations that occur during the snowmelt season.

3.3 Methodology

3.3.1 Field site and sampling

Field Site: A field camp was established within the Dark Zone, inland from Kangerlussuaq, during the spring (June 1 – July 1) of 2017, prior to the main melt. The camp was located approximately 30 km from the ice margin, near to the 'S6' weather station on the K-Transect (Fig 3.2A; 67°04'43.3" N, 49°20'29.7" W). The ablation of the snow surface was monitored, and snow, meltwater, ice core and snow covered surface ice samples were collected.

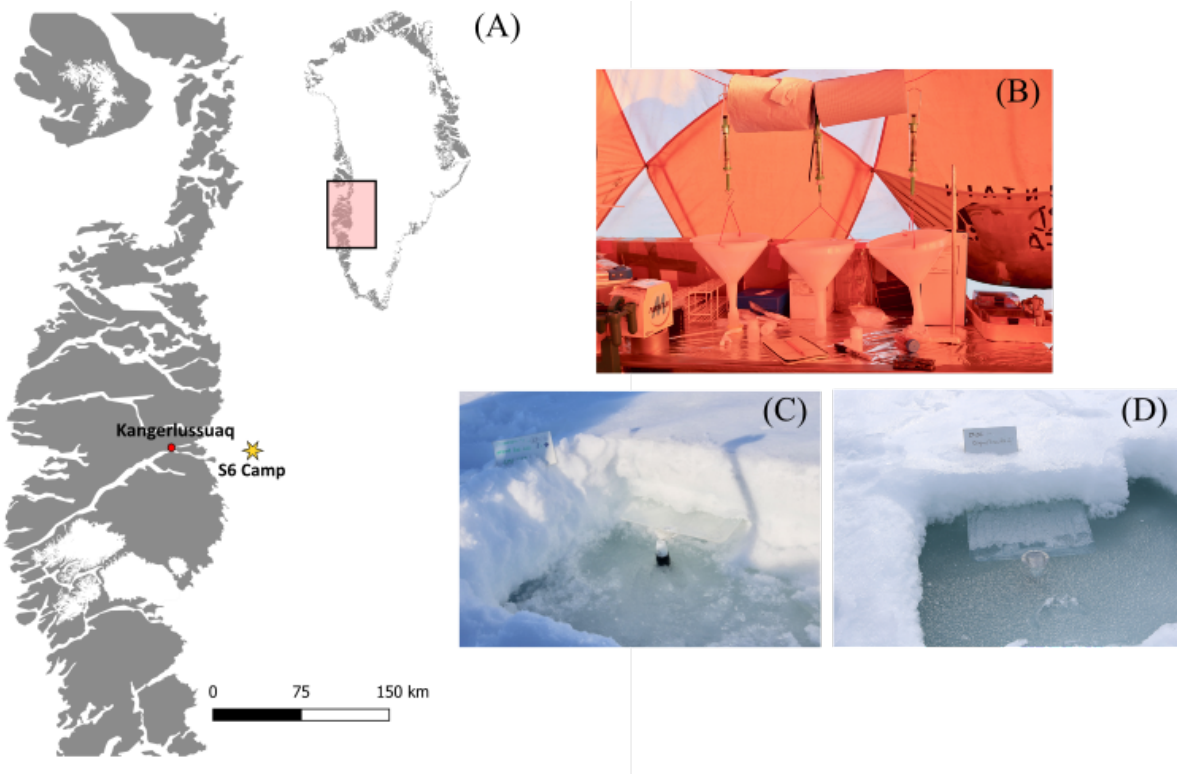

Figure 3.2: Camp location on the south-west margin of the GrIS (A), with inserts showing the crude laboratory snowmelt experiment (B), the *in-situ* snow pack meltwater collection (C), as well as the flooded *in-situ* snow pack meltwater (D).

Surface ablation monitoring: ablation was measured using 25 plastic ablation stakes, 20 of which comprised five quadrants of 5 × 5 m, drilled into ice around camp and throughout the sampling area. Measurements were taken every 1 – 3 days, from June 3 – 26 (Tedstone et al., 2020).

Snow sampling: throughout the field season, seven snow pits were dug using a pre-cleaned shovel in randomly selected locations upwind of camp. The snow cover was ~ 30 – 40 cm deep and contained 5 distinct layers, identified by hardness and crystal type. Samples of each distinctive layer were collected and transferred into sterile Whirl-pak™ bags using a pre-cleaned scoop. There was typically a coarse, loosely packed, base layer of facetted ice crystals, similar to depth hoar, topped by a basal ice layer some 3 – 4 cm thick. Although, ice crystals in the basal layer rounded when liquid water was present. A hard, coarse, tightly packed snow layer was found above the basal ice layer, containing ~ 2 – 3, thin (1 – 2 cm thick) ice layers interspersed throughout. Finally, the top of the snow pack consisted of a loose, coarse layer, topped by a freeze layer.

Snowmelt sampling: meltwater sampling was conducted daily from June 1 – 13, with rapid surface melting commencing around June 3. The rolling topography of the underlying ice resulted in areas where copious liquid water and slush ponded at the base of the snow pack in topographic lows soon after the onset of rapid melting (Fig. 3.2D). Even though meltwater flowed across the ice surface, there was sufficient surface melting to ensure that ponded meltwater remained at the base of the pack until June 13th. By contrast, topographic high spots were characterized by wet basal snow layers only, and excess water drained away across the ice surface.

Three snow pits were dug in a randomly selected sampling area upwind of camp. *In-situ* snowmelt water was collected by inserting a 38 × 49 × 3 cm pre-cleaned plastic tray at a slight upward angle at the base of the snow pack and securing a pre-cleaned 30 ml high-density polyethylene plastic bottle (Nalgene™) in the basal ice below the tray (Fig 3.2C). Meltwater from the snow pack collected on the tray and drained into the bottle. Bottles were changed daily or when full, depending on the rate of melt. Unfortunately, due to the rolling ice topography, described above, the bases of two of the snow pits flooded (Fig. 3.2D). Instead, the meltwater that pooled in these pits was collected daily by direct sampling of the *in-situ* meltwater into three, 30 ml pre-cleaned high-density polyethylene plastic bottle (Nalgene™) bottles.

Snowmelt elution experiments: a 10 × 10 cm section containing all the layers of each experimental snow pack, described above, was collected on June 1st using a pre-cleaned shovel and transferred into a sterile Whirl-pak™ bag. The sampled snow section was then homogenized in the Whirl-pak™ bag using the pre-cleaned shovel to break it apart then shaken and inverted to mix the snow. The homogenized sections were taken immediately to the laboratory tent where 500 g of each sample was transferred into pre-cleaned funnels. The funnel was then hung from a ring stand and a pre-cleaned 30 ml high-density polyethylene plastic bottle (Nalgene™) was placed underneath to collect the meltwater (Fig 3.2C). Bottles were replaced when full.

Surface Ice sampling: samples of the ice surface (n = 19) at the snow-ice interface were also collected throughout the field season (June 2 – 17) in random locations upwind of camp. First, the snow pack above the ice surface was removed, then the top ~ 2 cm of ice were sampled using a pre-cleaned ice saw and collected into a sterile Whirl-pak™ bag.

Ice core sampling: seven ice cores were collected throughout the field season using a 1 m Kovacs Corer in randomly selected locations upwind of camp. Fresh cores were laid out on clean tin foil where they were sectioned using a pre-cleaned hand saw. Distinct sections were determined by visible changes in ice structure and impurity loadings, for example changes in opacity, bubble content, cryoconite debris content and colour, with a typical core containing ~ 4 – 6 distinct sections.

3.3.2 Sample treatment and analytical methods

The methods described below are similar to methods described in chapter 2, section 2.3.4.1, yet due to subtle differences (i.e. major ion analysis) and for continuity throughout the thesis, the text has been left in its entirety.

Samples of snow and ice were returned to the field laboratory tent and left to melt overnight. All samples were analysed for dissolved inorganic and organic nutrients and major ions. Samples were filtered through a 25 mm × 0.22 μm cellulose nitrate inline syringe filter (Whatman™) and stored in two, 1.5 mL PP Autosampler vials for major ion analysis and a pre-cleaned 30 ml high density polyethylene plastic bottle (Nalgene™) for nutrient analysis. The vials for major ion analysis were chilled to the ambient air temperature of ~ 3 °C, while the Nalgene™ bottles for nutrient analysis were frozen immediately to a temperature of -20 °C using a Waeco 32 L Freezer. Samples were maintained at these temperatures during transport and in storage at the LowTex Laboratory at the University of Bristol. Nutrient samples were thawed immediately prior to analysis using a hot water bath set at a temperature of ~ 40 °C. Procedural blanks (n = 10) were collected during the course of the sampling season by processing deionized water in place of sample.

DIN species include NH_4^+, NO_2^- and NO_3^- and were quantified as NH_4^+, NO_2^- and total oxidised nitrogen (TOxN) ($NO_2^- + NO_3^-$) spectrophotometrically using a Gallery Plus Automated Photometric Analyzer (Thermo Fisher Scientific, UK). This combination of analysis allows the original NO_3^- concentration to be determined by subtracting NO_2^- from TOxN. The limit of detection (LoD) for all nutrients and major ions was determined by the mean concentration plus three times the standard deviation of procedural blanks (n = 6). TDN is the sum of DIN and DON and was determined by digesting the samples with a potassium persulfate, sodium hydroxide and boric acid reagent and autoclaving at 121 ºC for 30 minutes. This process causes the oxidation of organic nitrogen

compounds, which can then be measured as TOxN, as above. Measurements were based on the hydrazine-sulfanilamide reaction method measured at 540 nm. DON was then estimated by subtracting DIN from TDN (i.e. DON= TDN-DIN). LoDs were 2.4 μM (NH_4^+), 0.09 μM (NO_2^-) and 0.34 μM (TOxN and TDN/DON). Precisions were ± 2.1 % (NH_4^+), ± 0.49 % (NO_2^-) and ± 1.7 % (TOxN and TDN/DON), and accuracies were -1.8 % (NH_4^+), 0.16 % (NO_2^-) and -9.6 % (TOxN and TDN/DON), as determined from comparison with gravimetrically diluted 1000 mg L^{-1} NH_4^+-N, NO_2^--N and NO_3^--N certified stock standards to a concentration of 3.6 μM (NH_4^+ and NO_2^-) and 2.9 μM (TOxN and TDN/DON) (Sigma TraceCERT®).

DIP (dissolved inorganic phosphorus, principally PO_4^{3-}) was quantified using a Gallery Plus Automated Photometric Analyzer (Thermo Fisher Scientific, UK) using the molybdenum blue method measured at 880 nm. The LoD was 0.13 μM (PO_4^{3-}). Precision was ± 2.0 %, and accuracy was -8.3 %, as determined from comparison with gravimetrically diluted 1000 mg L^{-1} PO_4-P certified stock standards to a concentration of 1.61 μM (Sigma TraceCERT®).

Major ions (Cl^-, SO_4^{2-}, Na^+ and K^+) were quantified using capillary ion chromatography on a Thermo Scientific™ Dionex™ analytical ICS-5000, fitted with a simultaneous IonPac™ AS11-HC 2 × 250 mm anion-exchange column and an IonPac™ CS12 2 × 250 mm cation-exchange column. The LoDs were 0.18 μM (Cl^-), 0.03 μM (SO_4^{2-}), 0.62 μM (Na^+) and 0.38 μM (K^+). Accuracies were 4.3 % (Cl^-), -15 % (SO_4^{2-}), -17 % (Na^+) and 3.5 % (K^+). Precisions were ± 0.64 (Cl^-), ± 0.95 (SO_4^{2-}), ± 1.3 (Na^+) and ± 2.1 (K^+), as determined from comparison with a gravimetrically diluted single ion 1000 mg L^{-1} Fluka™ TraceCERT® ion chromatography standard to a concentration of 250 μg L^{-1} for each ion.

3.3.3 Snowmelt modelling

Simulations of meteorological and surface conditions were performed over S6 using version 3.9.6 of MAR, a regional climate model with a near-surface snow pack component (Fettweis et al., 2017). MAR uses an implementation of the detailed snow pack scheme, Crocus (Vionnet et al., 2012), coupled to its atmospheric scheme. Crocus simulates snow pack layering and metamorphism as a function of energy and mass transfer between the snow pack and the atmosphere, and the snow pack and the ground below. The snow pack is vertically discretized on a one-dimensional grid. The model was run on an equal-area 15 km resolution grid for the whole of the GrIS and was forced at its boundaries every 6 h by ECMWF ERA-Interim analysis (Dee et al., 2011). We utilized results from the pixel in the model domain, which was coincident with S6 and focused upon simulated density, temperature and liquid water content in the upper 0.65 m of the snow and ice layer, together with total meltwater production.

3.3.4 Data analysis

All measurements below the LoD were considered to be 0 for all statistical analyses. All DIN, DON, DIP and major ion data were water blank-corrected using values from the respective field procedural blanks resulting above the LoD. Additionally, all blank corrected values that were negative were assumed to be 0 for all statistical analyses. Statistical analysis was performed in RStudio v.1.1.414 (RStudio, Inc 2018). Non-parametric statistical analyses were used on all data after testing the homogeneity of variance and normality of distribution. Spearman's rank-order correlations were used to identify correlations between nutrient concentrations and time for snow meltwater samples. Identification of statistical differences in concentrations by date for snow pack meltwater and snow covered surface ice were achieved using Kruskal-Wallis rank sum tests, with post-hoc Dunn's test of multiple comparisons applied to all significant Kruskal-Wallis results. Data interpolation was performed on MAR snow pack data using the R packaged 'zoo' and the 'na.approx' function.

3.4 Results

3.4.1 Surface ablation

Quadrant 5 is representative of measurements taken from all 25 ablation stakes, and results are shown in Figure 3.3. The average melt rate calculated from all the poles over 23 days was ~ 2 cm day^{-1}. Surface melting was evident on June 1st and only patches of snow cover remained on June 26th.

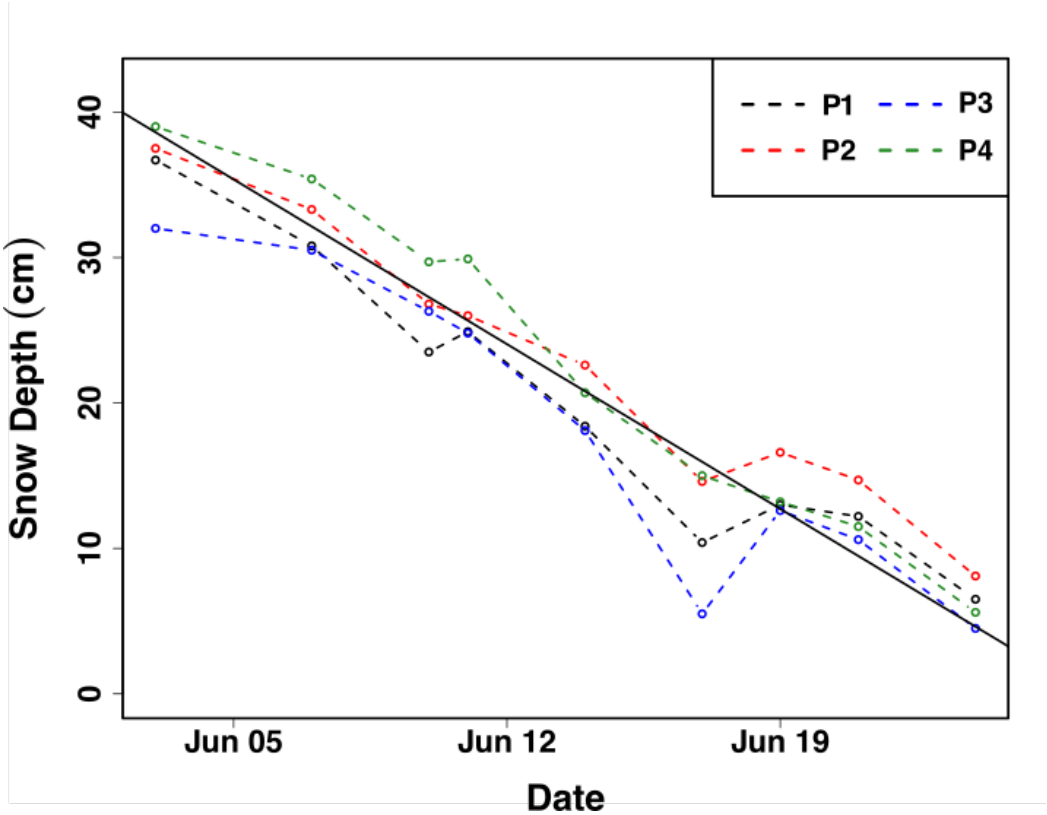

Figure 3.3: Representative surface ablation measurements during the sampling period. These particular results are from Quadrant 5, with an average ablation rate of ~ 1.6 cm day^{-1}, as given by the best fit linear regression line. P1 – 4 represent the four ablation poles comprising Quadrant 5.

3.4.2 Nutrients in the snow pack and underlying ice

DIP concentrations were seldom detected in any of the snow, ice and snowmelt samples (Table 3.1). Hence, the remainder of the paper deals exclusively with the concentrations of DIN and DON.

The composition of the snow pack, snow covered surface ice and underlying meteoric ice shows some striking differences. Average DIN concentration of the snow pack was 2.2 ± 0.3 µM, consisting mostly of NH_4^+ (1.0 ± 0.3 µM) and NO_3^- (1.1 ± 0.1 µM), with minimal NO_2^- (0.1 ± 0.0 µM). DON concentrations were relatively low, with an average concentration of 0.4 ± 0.1 µM (Table 3.1). Ice layers in the snow pack point to redistribution of both water and solute by melting, yet the concentration of solutes in the other snow layers show no clear and consistent pattern as a whole, with mean concentrations showing, at most, a variation of a factor of two. Hence the snow cover is relatively homogeneous in terms of solute distribution.

Snow covered surface ice was initially the most solute rich, before significant flooding at the snow-ice interface occurred, with an average DIN concentration of 4.7 ± 0.6 µM, consisting mostly of NH_4^+ (2.7± 0.5 µM) and NO_3^- (1.9 ± 0.4 µM), with minimal NO_2^- (0.1 ± 0.0 µM). DON concentrations

were lower, with an average concentration of 1.6 ± 0.6 µM (Table 3.1). The composition of 5 surface ice samples towards the end of the sampling season, after extensive melting had occurred, was lower. The average DIN concentration decreased significantly to 2.3 ± 0.7 µM ($\chi^2 = 4.9$, $p < 0.05$), still consisting mostly of NH_4^+ (1.4 ± 0.8 µM) and NO_3^- (0.8 ± 0.2 µM), with minimal NO_2^- (0.1 ± 0.1 µM), while the average DON concentration dropped to 0.9 ± 0.9 µM (Table 3.1), albeit insignificant.

Table 3.1: Mean ± SE concentration of N species ammonium (NH_4^+) nitrate (NO_3^-), nitrite (NO_2^-) and dissolved organic nitrogen (DON) in the snow pack, underlying surface ice and shallow meteoric ice (to a depth of ~ 1 m) near S6 on the K-Transect, SW Greenland. Dissolved inorganic phosphorus (DIP) data is also presented, although almost values are below quantification. *n* for snow pack samples denotes the number of layers sampled, *n* for snow covered surface ice samples denotes the number of samples taken on that date, and *n* for ice core samples denotes the number of ice layers, other than the surface 10 cm, which were sampled. Mean composition for snow covered surface ice samples only includes pre-melt sampling dates (02/06/17 & 04/06/17).

	NH_4^+(µM)			NO_3^- (µM)			NO_2^- (µM)			DIN (µM)			DON (µM)			DIP (µM)		
Snow Pack	mean	SE	*n*	mean	SE	*n*	mean	SE	*n*	mean	SE	*n*	mean	SE	*n*	mean	SE	*n*
Ice Layers	1.1	0.6	9	1.1	0.2	9	0.1	0.0	9	2.2	0.6	9	0.8	0.3	9	0.1	0.1	9
Top Snow layers	1.4	0.6	6	0.7	0.1	6	0.1	0.1	6	2.2	0.7	6	0.2	0.4	6	0.0	0.0	6
Mid Snow layers	0.7	0.7	4	1.7	0.7	4	0.1	0.1	4	2.5	0.7	4	0.0	0.0	4	0.0	0.0	4
Base Snow Layers	0.7	0.5	7	1.2	0.2	7	0.1	0.0	7	2.1	0.4	7	0.3	0.1	7	0.0	0.0	7
Mean Composition	1.0	0.3	26	1.1	0.1	26	0.1	0.0	26	2.2	0.3	26	0.4	0.1	26	0.0	0.0	26
Snow Covered Surface Ice (0 - 10 cm)																		
02/06/2017	2.2	1.2	4	1.1	0.2	4	0.0	0.0	4	3.3	1.0	4	0.3	0.2	4	0.0	0.0	4
04/06/2017	3.0	0.5	8	2.0	0.5	8	0.2	0.0	8	5.2	0.6	8	2.4	0.8	8	0.0	0.0	8
Pre-melt Mean Composition	2.7	0.5	12	1.9	0.4	12	0.1	0.0	12	4.7	0.6	12	1.6	0.6	12	0.0	0.0	12
17/06/2017	1.4	0.8	5	0.8	0.2	5	0.1	0.1	5	2.3	0.7	5	0.9	0.9	5	0.1	0.0	5
Shallow Meteoric Ice (11 cm - ~ 1 m)																		
03/06/2017	2.1	0.6	10	1.0	0.1	10	0.0	0.0	10	3.1	0.6	10	10.2	4.4	10	0.0	0.0	10
11/06/2017	2.3	1.2	3	0.5	0.3	3	0.0	0.0	3	2.8	0.9	3	1.2	0.6	3	0.0	0.0	3
21/06/2017	4.3	1.1	4	1.8	0.4	4	0.1	0.0	4	6.2	1.3	4	1.2	0.8	4	0.1	0.0	4
Mean Composition	2.6	0.5	17	1.1	0.1	17	0.0	0.0	17	3.8	0.6	17	6.5	2.8	17	0.0	0.0	17

The underlying shallow meteoric ice was richer in nutrients than the snow pack, but with lower concentrations than the snow covered surface ice. The average DIN concentration was 3.8 ± 0.6 µM, consisting mostly of NH_4^+ (2.6 ± 0.5 µM) and NO_3^- (1.1 ± 0.1 µM). By contrast, DON concentrations were the highest, with an average concentration of 6.5 ± 2.8 µM, although these concentrations are

variable. Elevated NH_4^+ concentrations were associated sections containing visible impurities, such as dispersed cryoconite, glacier algae and the particulates which they trap. Nutrient concentrations in the top ~ 10 cm of all ice cores were removed prior to calculation of the mean and standard error so as not to include the solute rich surface ice.

3.4.3 Snowmelt concentrations

The snowmelt elution experiments show the elution of solutes into the first meltwater fractions, as is commonly observed in other laboratory experiments (Figure 3.4). The fractionation factor is defined as the concentration of the ion in the particular melt fraction divided by the average concentration of the ion in all the melt fractions. Values above one show that the first melt fractions are enriched in solute.

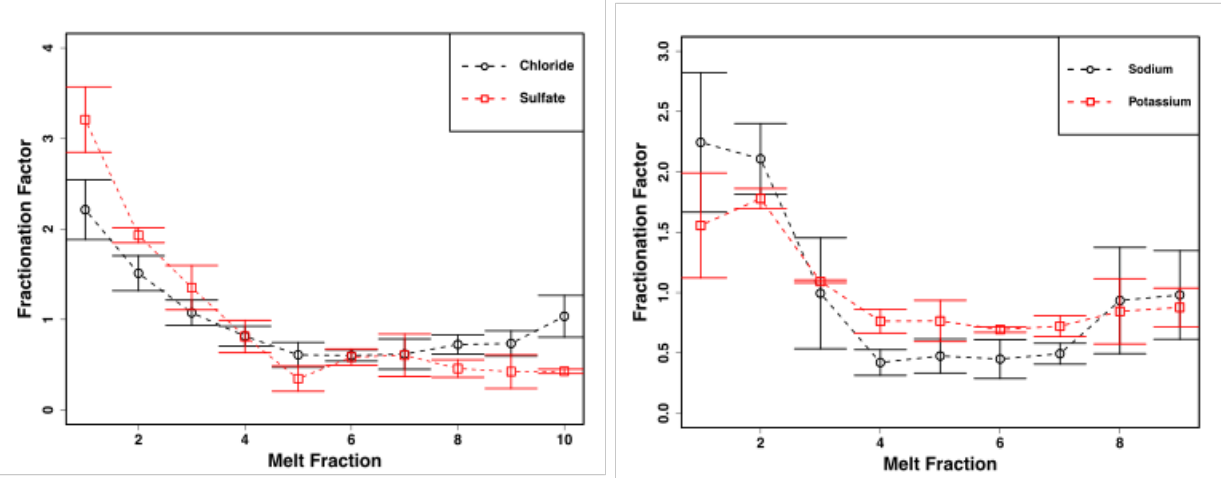

Figure 3.4: Mean ± SE chloride and sulphate concentrations (left panel) and sodium and potassium concentrations (right panel) from homogenized snow pack sections in crude laboratory melt experiment (Fig. 3.2C) (n = 3) demonstrate fractionation of solute into the first meltwater fractions.

NO_3^- concentrations are significantly higher in the first meltwater fractions ($\chi^2 = 19.0$, $p < 0.05$), with values of 3.2 ± 0.6 µM equivalent to a fractionation factor of ~ 3, and are significantly, negatively correlated to increasing melt fractions ($p < 0.00001$, $\rho = -0.7$) (Fig. 3.5). The latter meltwater fractions are more dilute, declining to concentrations of ~ 0.7 ± 0.1 µM in the last meltwaters.

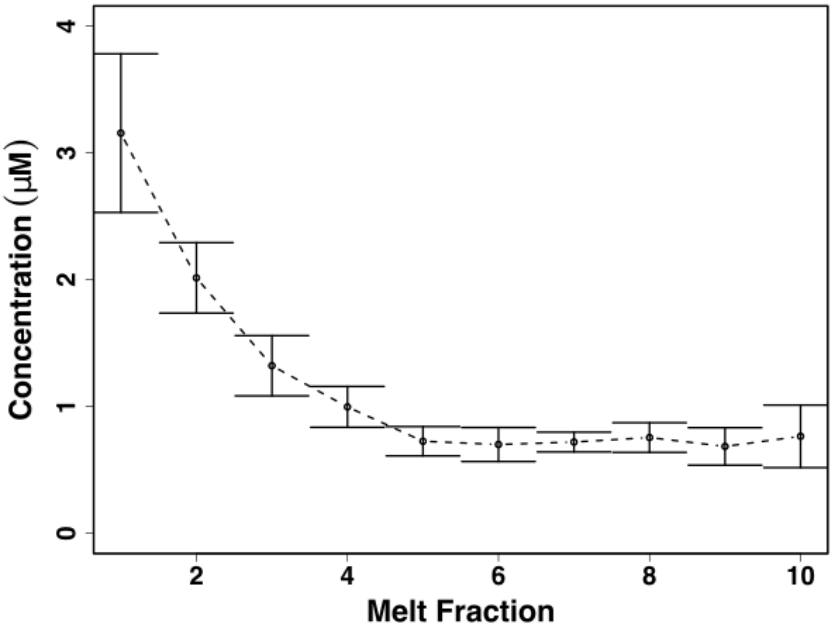

Figure 3.5: Mean ± SE nitrate concentrations from homogenized snow pack sections in crude laboratory melt experiment (n = 3) (Fig. 3.2C).

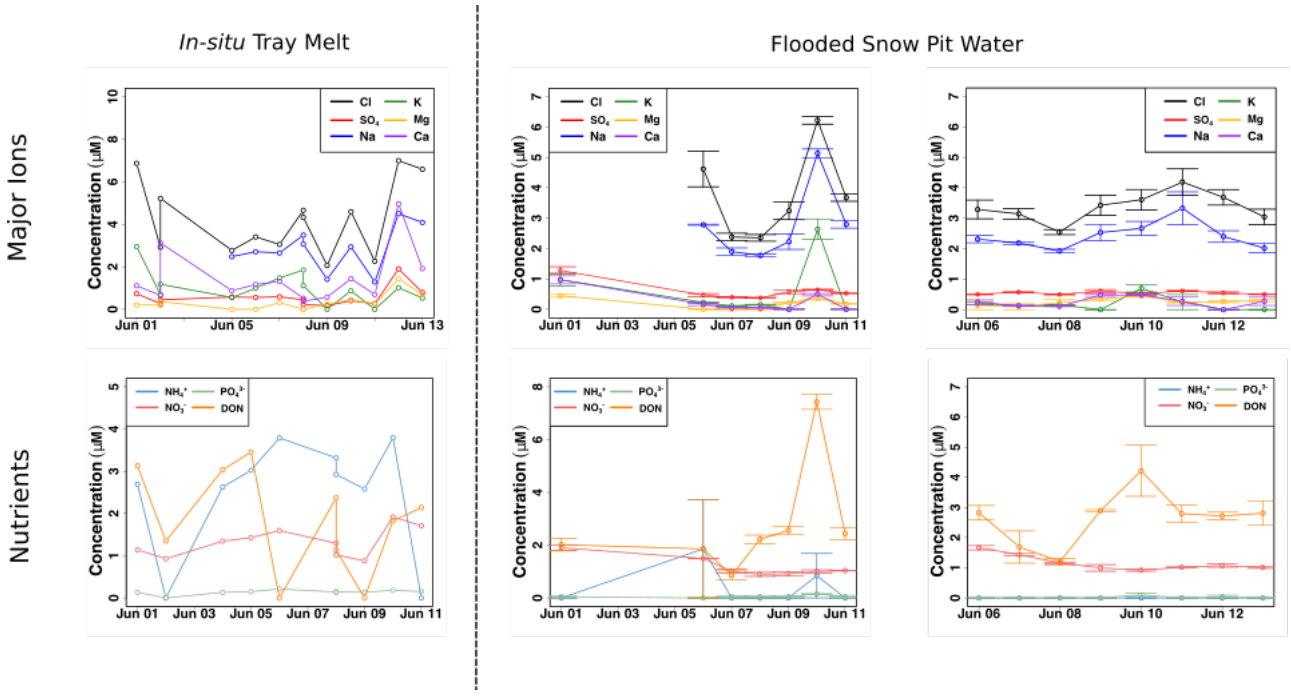

Figure 3.6: Far-left panel portrays meltwater concentrations of chloride (Cl^-), sulfate (SO_4^{2-}), sodium (Na^+), potassium (K^+), magnesium (Mg^{2+}) and calcium (Ca^{2+}) from the *in-situ* tray snowmelt (n = 1). The centre and far-right figures portray mean ± SE ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}) and dissolved organic nitrogen (DON) concentrations of the flooded snow pit meltwater (n = 2).

The NO_3^- concentrations in the snow pack, collected by the drip trays, are relatively constant, ~ 1 – 2 µM, similar to the average concentration of NO_3^- in the snow pack (Fig. 3.6). NH_4^+ and DON

concentrations, however, fluctuate greatly, from 0 – 4 µM for both, with the higher concentrations well in excess of those found in the snow pack. The flooded snow pit waters show some differences, with significantly higher initial concentrations of NO_3^- (~ 2 µM), before declining to ~ 1 µM (χ^2 = 24.8, $p < 0.001$), resulting in a significant, negative correlation over time ($p < 0.0001$, $\rho = -0.6$). DON concentrations are more variable and range from ~ 2 µM to 7 µM, with concentrations significantly higher towards the end of the sampling period than during the onset of melt ($\chi^2 = 25.4$, $p < 0.001$), resulting in a significant, positive correlation over time ($p < 0.01$, $\rho = 0.4$). In marked contrast, concentrations of NH_4^+ are usually below detection, and could only be detected in two samples, where concentrations were ~ 2 and ~ 1 µM (Fig. 3.6).

3.4.4 Modelled meltwater production during transition from snow to ice

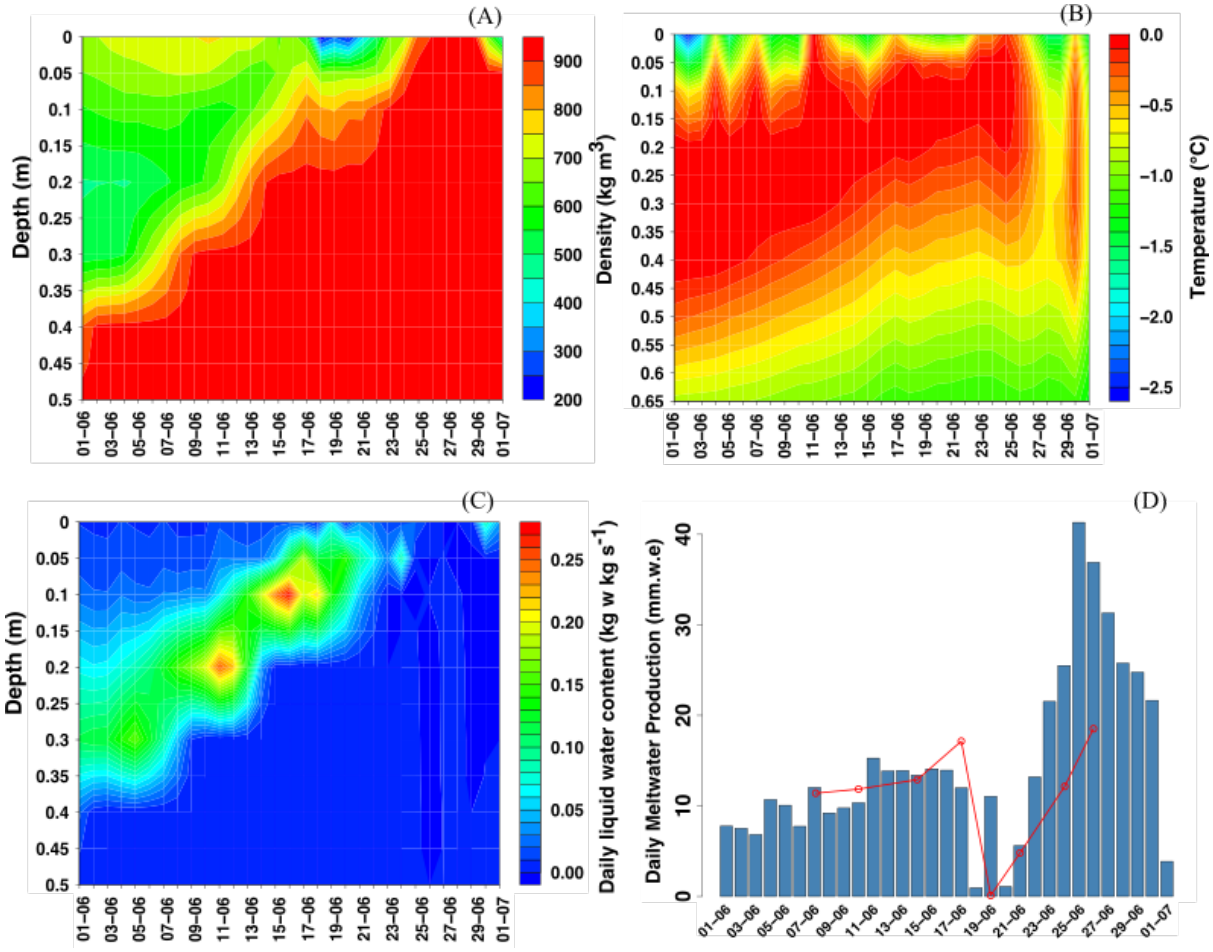

Figure 3.7: Modelled snow and ice density (A), temperature (B), liquid water content (C) and daily meltwater production (D), as calculated by MAR in the vicinity of S6 on the K-Transect, SW Greenland, from June 1 – July 1. The red line on plot (D) represents ground truthed daily meltwater production calculated using Quadrant 5 daily snow pack melt rate multiplied by an average snow density of 0.6 kg m^3 determined from plot (A).

MAR calculated an initial snow pack depth of ~ 40 cm on June 1st (Fig. 3.7A; ice density is ~ 920 kg m^3 and is thus represented in red), which is consistent with our field observations, given the underlying rolling topography of the surface ice. The temperature of most of the snow pack and snow covered surface ice (to a depth of at least 10 cm) are calculated to be 0 °C for most of the sampling period (Fig. 3.7B). MAR predicts that the snow pack already contains liquid water on June 1st, consistent with our collection of melt within the snow pack. The presence of liquid water in the snow pit and the flooding of basal snow in ice topographic lows is consistent with the model output (Fig. 3.7C). Finally, the calculated daily meltwater production is ~ 10 mm w.e. during the snow pack sampling season (Fig. 3.7D). Our average surface lowering was of the order of 1.9 cm day^{-1}, which, when multiplied by the MAR calculated snow density of ~ 0.5 – 0.6 kg cm^{-3} (Fig. 3.7A), is equivalent to a daily production of ~ 10 mm w.e. Furthermore, ground truthed data using daily snow pack melt rates from Quadrant 5 multiplied by an average snow density of 0.6 kg cm^{-3} (Fig. 3.7A) follows the trend of daily meltwater production calculated by MAR (Fig. 3.7D).

3.5 Discussion

This paper is the first to document N concentrations in snowmelt during the spring thaw on the GrIS. The snow pack that was sampled on June 1st had already been partially melted, as was evident from the ice layers both within, and near the base of the snow pack. The average NO_3^- concentration of the snow pack, 1.1 µM (Table 3.1), is lower than those found in the interior of the ice sheet (Fibiger et al., 2016), but comparable to those nearer the margin (Telling et al., 2012b). It is equivocal whether the slightly elevated average concentrations of NO_3^- contained in the surface ice (Table 3.1), 1.9 µM, with respect to the underlying shallow meteoric ice, 1.1 µM, is the result of refreezing of earlier solute-rich meltwater. However, superimposed ice formation, when meltwaters refreeze on the underlying cold ice surface, is common on Greenland (Huybrechts et al., 1991), and so is a plausible explanation. A difficulty with this explanation is that DON and NH_4^+ concentrations in both the surface ice (mean values 1.6 and 2.7 µM, respectively) and shallow meteoric ice are high (mean values 6.5 and 2.6 µM, respectively) in comparison with average snow pack concentrations (mean values 0.4 and 1.0 µM, respectively; Table 3.1). Our field observations of the nature of the surface ice following coring were that it was difficult to be certain that it was truly superimposed ice. Instead, the surface ice, although relatively bubble free, appeared to form a continuum with the previous season's weathering crust, potentially via early concentrated snowmelt soaking into and refreezing within the former weathering crust (Fig. 3.8). Therefore, it may well be that the surface ice was indeed a mixture of both superimposed ice and weathering crust. DON concentrations are discussed further below.

The average ablation rate recorded during the measuring period, from June 3 – 26, was 1.9 cm day^{-1}, resulting in copious meltwater flowing both through the snow pack and across the snow-ice interface,

with MAR calculating ~ 10 mm w.e. day^{-1} produced during the snowmelt sampling period, from June 1 – 26 (Fig. 3.7D). The concentration of NO_3^- in snowmelt collected within the snow pack was relatively constant, with values oscillating around the mean snow pack concentration (1.1 μM; Fig. 3.6), and tracked the changes in major ion concentrations to a first approximation (Fig. 3.6). This suggests that NO_3^- in snowmelt to be associated with factors such as variations in melt rate, melt routing and solute banding in the snow path, which control the overall composition of more conservative solutes in snow and snowmelt, such as Cl^- (Tsiouris et al., 1985; Cragin et al., 1996). NH_4^+ and DON concentrations, however, were much more variable, with both exceeding the average snow pack concentration by factors of ~ 3 and ~ 10, respectively, while also having values below quantification. It is therefore possible that microbiological processes in the melting snow pack may be influencing the concentrations of NH_4^+ and DON. NH_4^+ is oxidized by several types of microbes in many oxic environments (Venter et al., 2004; Könneke et al., 2005; He et al., 2018) and both NH_4^+ and DON are common by-products of microbial activity (Stibal et al., 2012a; Telling et al., 2012b). Hence, microbial communities within the melting snow pack may have an impact on the oscillating concentrations of NH_4^+ and DON.

The crude laboratory melt experiments show that even the leached, well metamorphosed snow found in the snow pack during early June exhibits elution of solutes, including NO_3^-, during melting (Figs. 3.4 and 3.5). This is in marked contrast to the more constant NO_3^- concentrations found in the snow pack meltwaters. The most likely explanation for this is that, stochastically, meltwater leaching off the surface layers of solute-rich ice crystals is approximately balanced by the melting of solute-poor ice crystal interiors, so that, on average, the snowmelt composition is similar to the average composition of the snow pack. This is unlikely to be the case earlier in the melt season, when the first meltwaters draining from snow packs are often solute-rich (Tranter et al., 1986; Cragin et al., 1996; Hodson, 2006).

The nutrient composition in meltwaters that flooded the base of the snow pits was very different to that in the snow pack (Fig. 3.6). This is an important observation for those designing glacier algae incubation studies and modelling the potential for glacier algal productivity in the early melt season. Initial NO_3^- concentrations were significantly higher than concentrations later in the sampling period, reaching as high as ~ 2 μM, nearly double the average snow pack concentration, then decreasing and stabilizing around 1 μM, similar to the average snow pack concentration, for the remainder of the sampling period. DON concentrations fluctuated significantly, with initial concentrations of ~ 1 μM, then later reaching as high as 7 μM. By contrast, NH_4^+ concentrations are mostly undetected. The pre-melt snow covered surface ice contained comparable concentrations of NO_3^-, 1.9 μM, making it a likely source of the additional flooded meltwater NO_3^-, while both the shallow meteoric ice and

surface ice are the most likely DON sources. As high DON concentrations, between 5 – 14 μM, have been reported for surface ice hosting high concentrations of glacier algae in the GrIS Dark Zone during the summer months (Holland et al., 2019), it is likely that DON frozen into the weathering crust during the previous fall is a source to meltwater flowing across the snow-ice interface. The lack of NH_4^+ in the flooded basal meltwaters, in contrast with the relatively high average concentrations in the surface and shallow meteoric ice, ~ 2.6 μM, points to microbial processes being a sink near the snow-ice interface. Our field observations suggest that, within the top 10 cm of ice, there might be a mixed transition layer of bubble-free surface ice, consistent with the presence of superimposed ice, through to the frozen weathering crust of previous year (Fig. 3.8), as described above. Hence, we postulate that nutrients from the superimposed ice and the previous season weathering crust are leached as snowmelt floods and partially melts the surface ice. Evidence for the leaching of nutrient from the surface ice is shown by a significantly lower DIN concentration, the lowest NO_3^- and NH_4^+ concentrations and lower than average DON concentrations (Table 3.1) in snow covered surface ice collected on June 17th, which had been subject to copious wetting by snowmelt. NO_3^- concentrations from snow covered surface ice collected on June 17th were similar to those reported for ice collected in June on a transect through the GrIS Dark Zone (~ 0.6 μM), though the study makes no mention of the presence of snow on top of the ice (Telling et al., 2012b). The inverse correlations between NO_3^- and DON concentrations over time in the meltwater traversing the snow-ice interface further supports the potential leaching of surface ice, as NO_3^- rich early snowmelt ice likely melted initially, attributing to significantly higher early NO_3^- concentrations, resulting in the exposure of the DON rich previous season weathering crust and eventual shallow meteoric ice, resulting in the late significant increase in DON (Fig. 3.8).

Shallow meteoric ice results show the top ~1 m of ice to be nutrient loaded, with high concentrations of both DIN and DON (Table 3). This is consistent of nutrient transport within the weathering crust during the previous ablation season that were likely frozen in at the onset of winter. We show this ice layer to be a nutrient source for developing glacier algal blooms during the initiation of the spring thaw. The fate of these nutrients during the ablation season, however, cannot be stated unequivocally. It is likely that as the surface ice ablates over the season, nutrients are released to the established glacier algal bloom as a further nutrient source. As the ablation season progresses, however, and the ice surface is removed, the depth of the weathering crust likely increases in concert, leading to a relatively constant 1 – 2 m thick, nutrient charged layer, which may be frozen in over winter and again act as a nutrient source to the following season glacier algal bloom. Yet, transport downstream is also possible during large scale melt events, which have been shown to strip the weathering crust (Tedstone et al., 2017), potentially leading to large pulses of nutrients transported from the supraglacial environment as opposed to a constant source.

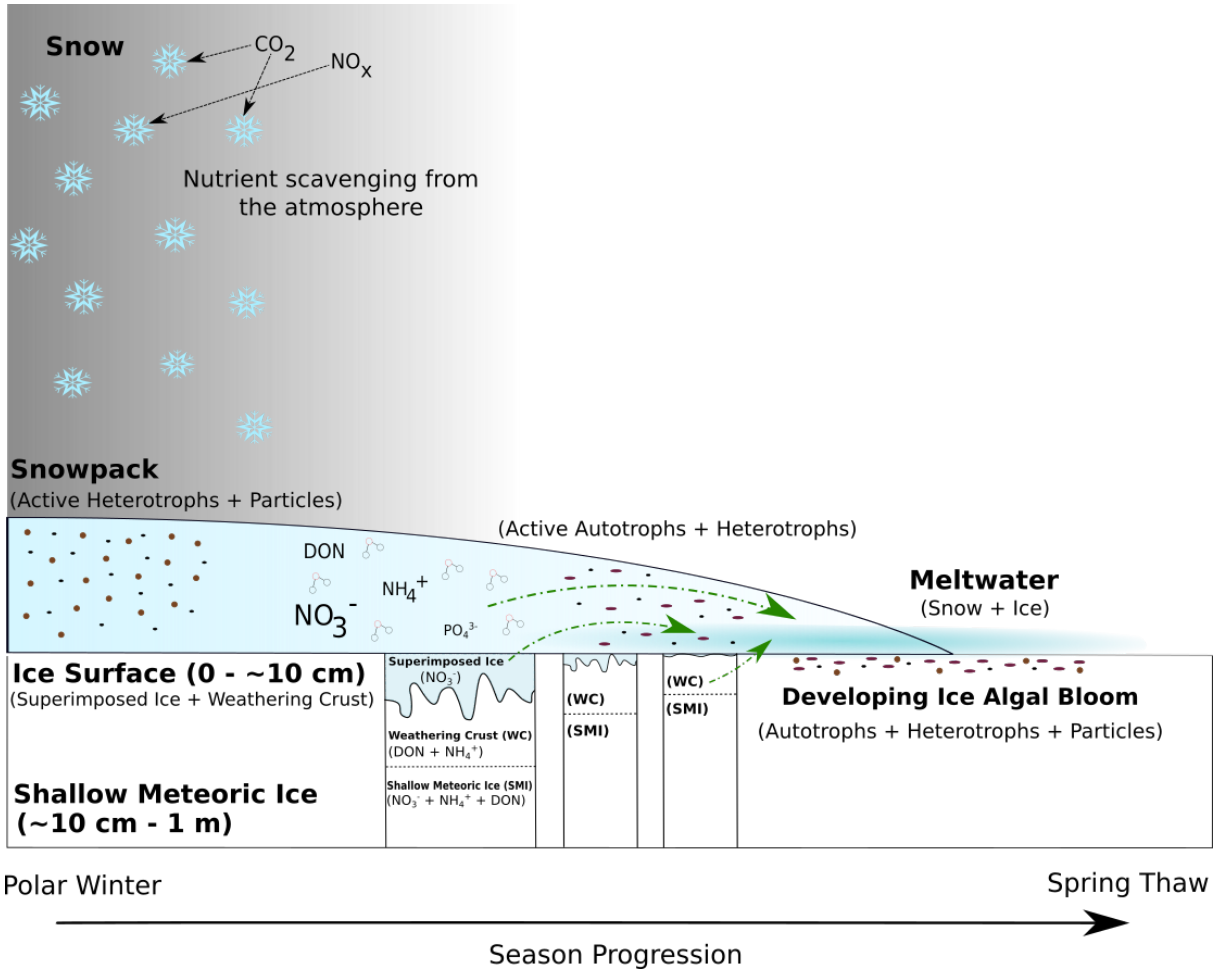

Figure 3.8: Conceptual diagram of the transition from polar winter to spring thaw in the supraglacial environment of the GrIS Dark Zone. Green lines represent nutrient inputs to surface ice environments during the spring thaw. Active microbial communities represent potential influencers on nutrient concentration and phase released by the different sources.

A key observation from the MAR modelling is that at least 10 cm of the surface ice underlying the snow pack had a temperature of 0 °C (Fig. 3.7A), likely warmed by factors such as superimposed ice formation (Wadham and Nuttall, 2002) and the adsorption of solar radiation from light penetrating the shallow snow cover. The latter will be further absorbed by dark impurities, including glacier algae, frozen into the weathering crust of the previous year, which may result in localized melting of the ice, similar to the frozen cryoconite holes in the McMurdo Dry Valleys, Antarctica, melting out first at depth in the ice (Bagshaw et al., 2007). This concurs with field observations of the shallow meteoric ice containing wet layers in the presence of glacier algae and dispersed cryoconite. Hence, we contend that the MAR modelling results, supported by our field observations, are evidence for the production of micro-environments in ice beneath the thinning, melting snow cover in which glacier algae can begin to metabolise before the snow cover is fully ablated (Fig. 3.1).

3.6 Conclusion

This study provides the first preliminary dataset on the concentration and speciation of dissolved N in snowmelt within the Dark Zone of the GrIS. Comparison of the concentrations of the same species in the associated snow pack, surface ice beneath the snow pack and shallow meteoric ice beneath it provides an insight into the physical and biological processes that control the abundance and species. We contend that, as snowmelt has been shown to be an important nutrient source to many other cryospheric environments, knowledge regarding its impact on the seasonal growth cycle of glacier algae is critical. Melting had already occurred before sampling commenced as evidenced by observed ice layers in and near the base of the snow pack and the possible formation of superimposed ice via earlier snowmelt soaking into and refreezing within the weathering crust of the previous summer. The decaying, partially leached snow pack gave rise to meltwaters in which the NO_3^- concentration was relatively constant and approximated the average NO_3^- concentration of the snow pack (1.1 μM). By contrast, NH_4^+ and DON concentrations were more variable, sometimes fluctuating notably higher and lower than their average snow pack concentration, 1.0 and 0.4 μM respectively. This variability could be the result of microbial uptake and production within the melting snow pack. Snowmelt flooding the snow-ice interface appears to scavenge DON and NO_3^- from the weathering crust, superimposed ice continuum, yet NH_4^+ is largely absent, presumably as a result of consumption by microbial activity. Absorption of solar radiation by dark particulates, or light adsorbing impurities, including glacier algae, likely result in localized melting and production of micro-environments in which the glacier algae can metabolise before the surface snow cover disappears. The assertion is consistent with field observations of localized areas of melt around debris-rich layers in the shallow meteoric ice cores and is supported by modelling results from MAR, which calculate that at least 10 cm of the surface ice below the snow pack is at 0 °C. Hence, glacier algal blooms may commence before the snow pack has completely melted (Fig.3.1) and occur at a time when meltwaters percolating into the remains of the weathering crust from the previous melt season are enhanced in nutrients, as a result of scavenging from the snow pack, superimposed ice layers and the former weathering crust itself. These processes should be considered when defining incubation experiments which aim to understand the initiation of glacier algal blooms.

Chapter 4: Dissolved organic nutrients dominate melting surface ice of the Dark Zone (Greenland Ice Sheet)

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> **Holland, A. T.**, Williamson, C. J., Sgouridis, F., Tedstone, A. J., McCutcheon, J., Cook, J. M., Poniecka, E., Yallop, M. L., Tranter, M., and Anesio, A. M. (2019). Dissolved organic nutrients dominate melting surface ice of the Dark Zone (Greenland Ice Sheet), *Biogeosciences*, 16, 3283-3296, https://doi.org/10.5194/bg-16-3283-2019.

Martyn Tranter, Alex Anesio, Marian Yallop and I designed the study. Samples were collected by me, Chris Williamson, Martyn Tranter, Alex Anesio, Andrew Tedstone, Jennine McCutcheon, Joe Cook and the Black & Bloom group collected the samples. Chris Williamson provided algal counts for the mid to late ablation periods. I conducted all the nutrient analysis and was aided by Fotis Sgouridis in the instrument maintenance and data analysis. The manuscript was written by me with inputs from Martyn Tranter, Chris Williamson, Andrew Tedstone and Alex Anesio. All authors reviewed the final manuscript.

4.1 Abstract

Glaciers and ice sheets host abundant and dynamic communities of microorganisms on the ice surface (supraglacial environments). Recently, it has been shown that Streptophyte glacier algae blooming on the surface ice of the south-western coast of the Greenland Ice Sheet are a significant contributor to the 15-year marked decrease in albedo. Currently, little is known about the constraints, such as nutrient availability, on this large-scale algal bloom. In this study, we investigate the relative abundances of dissolved inorganic and dissolved organic macronutrients (N and P) in these darkening surface ice environments. Three distinct ice surfaces, with low, medium and high visible impurity loadings, supraglacial stream water and cryoconite hole water, were sampled. Our results show a clear dominance of the organic phase in all ice surface samples containing low, medium and high visible impurity loadings, with 93 % of the total dissolved nitrogen and 67 % of the total dissolved phosphorus in the organic phase. Mean concentrations in low, medium and high visible impurity surface ice environments are 0.91, 0.62 and 1.0μM for dissolved inorganic nitrogen (DIN), 5.1, 11 and 14μM for dissolved organic nitrogen (DON), 0.03, 0.07 and 0.05μM for dissolved inorganic phosphorus (DIP) and 0.10, 0.15 and 0.12 μM for dissolved organic phosphorus (DOP), respectively. DON concentrations in all three surface ice samples are significantly higher than DON concentrations in supraglacial streams and cryoconite hole water (0 and 0.7μM, respectively). DOP concentrations are higher in all three surface ice samples compared to supraglacial streams and cryoconite hole water (0.07μM for both). Dissolved organic carbon (DOC) concentrations increase with the amount of visible impurities present (low: 83μM, medium: 173μM and high: 242 μM) and are elevated compared to supraglacial streams and cryoconite hole water (30 and 50μM, respectively). We speculate that the architecture of the weathering crust, which impacts on water flow paths and storage in the melting surface ice and/or the production of extracellular polymeric substances (EPS), containing both N and P in conjunction with C, is responsible for the temporary retention of DON and DOP in the melting surface ice. The unusual presence of measurable DIP and DIN, principally as NH_4^+, in the melting surface ice environments suggests that factors other than macronutrient limitation are controlling the extent and magnitude of the glacier algae.

4.2 Introduction

There has been a significant increase in the net mass loss of the Greenland Ice Sheet (GrIS) during the past two decades (Rignot and Kanagaratnam, 2006; Rignot et al., 2011; Shepherd et al., 2012), from 34 Gt yr^{-1} to 215 Gt yr^{-1} between 1992 and 2011 respectively (Sasgen et al., 2012). Surface melt is the primary driver for the increase in ice mass loss (~ 68 %) since 2009, with the remaining (~ 32 %) coming from solid ice discharge or calving (Enderlin et al., 2014). There are two major reasons for this marked increase in surface melting. First, the extent of bare, melting surface ice increased, on average, by 7158 km^2 per year from 2000 to 2014 (Enderlin et al., 2014; Shimada et al., 2016).

Second, the albedo of bare surface ice areas declined between 2000 and 2012, with south-west Greenland exhibiting the greatest decrease of up to 18 % (Box et al., 2012). A persistent Dark Zone in this region, some 20 – 30 km inland and ~ 50 km wide, has reoccurred annually since at least 2001 (Wientjes and Oerlemans, 2010; Box et al., 2012; Stroeve et al., 2013; Tedstone et al., 2017). There is significant variability in the annual extent of the Dark Zone (Shimada et al., 2016), which may be the result of both inter-annual climatic variability and factors associated with the ice surface, such as melt-out of ancient Holocene dust particles (Wientjes et al., 2011; Tedstone et al., 2017).

Both snow and bare ice albedo are reduced by light absorbing impurities (LAIs), of both biological and mineralogical origin (Gardner and Sharp, 2010), which include atmospheric dust and black carbon, cryoconite, and particulates within the meteoric ice that melt out during the ablation season (Warren and Wiscombe, 1980; Warren, 1984; Warren and Wiscombe, 1985; Gardner and Sharp, 2010; Wientjes et al., 2012; Cook et al., 2016b). The importance of biological LAI, particularly Streptophyte glacier algae, which bloom in surface ice environments during summer ablation seasons, as a factor in albedo decline has been identified in recent years (Yallop et al., 2012). The effect has become known as "bioalbedo", which is derived from the original term "biological albedo reduction" (Kohshima et al., 1993; Cook et al., 2017a). Bioalbedo is attributed to a combination of the high abundance of cells that grow during the bloom (up to ~ 10^4 cells ml^{-1} surface ice) and the heavily pigmented nature of ice algal cells, which include a unique dark UV-VIS absorbing pigment, purpurogallin, that provides photo-protection from the extreme solar radiation in supraglacial environments (Remias et al., 2012b; Williamson et al., 2018). Tedstone et al., (2017) concluded that ice algal blooms are the main factor responsible for inter-annual variability in the extent, magnitude and duration of the Dark Zone, which seem to be regulated by climatic drivers, including the June-July-August sensible heat flux anomaly and the timing of snow-line retreat. The spatial extent of ice algal blooms may also be linked to the availability of mineralogic LAIs, such as late Holocene dust particles melting out of the meteoric ice (Wientjes et al., 2012). However, the linkage between particles and algae is not presently understood (Tedstone et al., 2017).

C, N and P are essential for all living organisms, providing the basis for cellular mass and all metabolic activity (Redfield et al., 1963; Hessen et al., 2013). Carbon is usually in ready supply in surface ice environments, both from the atmosphere and from bubbles trapped in snow and ice, and so nitrogen and phosphorus are more likely the limiting factors for growth and activity of microorganisms (Stibal et al., 2009; Lutz et al., 2017). Bioavailable forms of N are less readily available, being largely confined to NO_3^- and NH_4^+ in dry and wet deposition from the atmosphere (Wolff, 2013), and from snow- and ice-melt (Telling et al., 2011). Dissolved inorganic phosphorous (DIP) is typically the least available nutrient in supraglacial environments, since it is a largely rock-

derived and is only released by chemical weathering or bio-mining (Stibal et al., 2008b; Stibal et al., 2009) . P sources in remote glaciated environments, such as the Dark Zone, are largely confined to the small quantities of particulates deposited from the atmosphere and the melt out of debris in snow and ice (Wientjes and Oerlemans, 2010).

The presence of such large-scale algal blooms in the Dark Zone might suggest that these environments are nutrient-rich. This would contrast with the current literature, which suggests that supraglacial environments in the Dark Zone, similar to those found in Svalbard, the margins of the Greenland Ice Sheet and Antarctica, are extremely oligotrophic (Stibal et al., 2008b; Stibal et al., 2009; Telling et al., 2011; Telling et al., 2012b; Bagshaw et al., 2013; Hawkings et al., 2016; Wadham et al., 2016). Mean dissolved inorganic nitrogen (DIN) concentrations in Greenland ice are ~ 1.4 μM, with NO_3^- and NH_4^+ composing 0.97 μM and 0.39 μM, respectively (Wolff , 2013). There are relatively few measurements of nutrient concentrations in the surface ice environments of the Dark Zone (Telling et al., 2012b; Wadham et al., 2016), but the average NO_3^- concentration in surface ice along the K-Transect east of Kangerlussuaq, which passes through the Dark Zone, has been reported to be 0.6 ± 0.1 μM between 17-79 km from the ice sheet margin (Telling et al., 2012b), while DIP concentrations were below the detection limit, 0.33μM P (Telling et al., 2012b). DIN concentrations in snow sampled before the start of the ablation season at the margin of the GrIS had higher concentrations, with an average of 1.4 μM (Telling et al., 2012b), similar to those of Wolff (2013). Hence, there is no real evidence that neither N nor P concentrations in snow and ice sampled in the vicinity of the Dark Zone are higher than for average Greenland ice. The relatively low concentrations of macronutrient in the snow and ice of the SW Greenland Ice Sheet means that algal blooms are likely to rapidly sequester N and P from snow and ice melt, particularly as the blooms reach their zenith at the height of the ablation season. For example, NPP (Net Primary Production) values in the wet, melting surface ice (also called rotten ice, or the weathering crust) during blooms range from 21 – 100 μmol C l^{-1} day^{-1} (Chandler et al., 2015; Williamson et al., 2018). Should the mean DIN concentration of the ice melt be 1.4 μM, this implies a C:N molar ratio of 15 – 71 if all the DIN is sequestered into new organic matter and no other sources of DIN are present. There is no readily available C:N ratio of glacier algae in the literature, but typical C:N ratios of sea ice algae are in the range of 12-46 (Niemi and Michel, 2015). It is even more difficult to find C:N:P ratios of glacier algae, but should the C:P ratio be in the region of 100:1 to 1000:1, the P demand will be 0.02 – 1 μM.

Blooms in other aquatic ecosystems are associated with efficient recycling of nutrients when new sources of N and P are in scarce supply, often with a balance between nutrient uptake and remineralization (Dodds, 1993), allowing nutrient accumulation in biomass over time. This balance

does not appear to arise in the surface ice environments of other High Arctic and polar glaciers studied to date. These are predominantly in cryoconite holes, which are water-filled cylindrical holes with an organic-rich basal sediment in the ice surface that host a range of microbes, including cyanobacteria (Christner et al., 2003; Anesio and Laybourn-Parry, 2012; Telling et al., 2012b). Dissolved macronutrients tend to become concentrated in organic phases (Stibal et al., 2008b; Telling et al., 2014), suggesting an imbalance in the uptake and remineralization of dissolved inorganic nutrients in cryoconite hole environments. Indeed, the only ratio of primary production to remineralization measured in the Dark Zone is 30:1 (Nicholes et al., 2019). To date, dissolved organic nitrogen (DON) concentrations in the Dark Zone have only been reported in two studies (Telling et al., 2012b; Wadham et al., 2016), but neither focus on ice populated by Streptophyte glacier algae. Telling et al., (2012) reported a near 1:1 relationship between NO_3^- and total dissolved nitrogen (TDN), suggesting that DON comprised only a small portion of the TDN pool in snow and ice samples. By contrast, Wadham et al., (2016) suggested mineralization of organic matter by microbial activity, either within the cryoconite holes themselves or in debris- and cryoconite-rich "dirty" surface ice contributed to DON concentrations that could reach 0.7 μM and 3.0 μM, respectively. No dissolved organic phosphorous (DOP) concentrations in the surface ice environments in the Dark Zone have been reported to date.

Several studies have noted the heterogeneity in the spatial distribution of glacier algae in the melting surface ice of the Dark Zone (Yallop et al., 2012; Williamson et al., 2018). This heterogeneity occurs on length scales of cm to 10s of m (Yallop et al., 2012). This might well signify that macronutrient concentrations are also variable on this scale, yet no studies to date have examined variability on these length scales. We contend that it is important to determine the concentrations and relative proportions of dissolved inorganic and organic nutrients in melting surface ice environments of Dark Zone, particularly during Streptophyte glacier algae blooms, since a knowledge of DIN, DON, DIP and DOP may be crucial to better understand how glacier algae and bacteria can retain, utilize and recycle their limited nutrients to sustain the large-scale blooms observed in this region of the Greenland Ice Sheet. The aims and objectives of this study, therefore, are threefold. First, we aim to quantify dissolved nutrient concentrations in the supraglacial environments of the Dark Zone during the peak ablation season. Second, we determine the relative abundance of dissolved inorganic and organic nutrients during the peak ablation season when microbial recycling is likely to have the greatest influence on the dissolved inorganic and organic ratios. Finally, we investigate if there are systematic changes in the relative proportions of dissolved macronutrients during increased colonization of melting surface ice, which might shed light on the limiting nutrient on algal blooms.

Figure 4.1: Map showing location of Camp BLACK & BLOOM 2016 (67°04'43.3"N, 49°20'29.7"W). Background image sourced from Sentinel 2, taken on 26/7/2016.

4.3 Methodology

4.3.1 Field site and sampling

A field camp was established within the Dark Zone, adjacent to Kangerlussuaq, during the summer of 2016. The camp was located approximately 30 km inland from the ice margin, near to the 'S6' weather station on the K-transect (Fig 1; 67º04'43.3" N, 49º20'29.7" W). Samples were collected from a designated area of approximately 500 × 500 m, which included surface ice, supraglacial stream and cryoconite hole habitats. Sampling occurred at intervals of approximately three days from 15th of July to 14th of August 2016. A categorical sampling strategy was employed, given the evident spatial heterogeneity apparent in ice algal distributions. Five different habitats were randomly sampled; melting surface ice with three differing amounts of visible impurities, referred to here as surface ice with "low" (n=19), "medium" (n=19), and "high" (n=19) visible impurities (Fig. 4.2) (Yallop et al., 2012), water from supraglacial streams (n=10) and cryoconite hole water (n=14). Supraglacial stream and cryoconite hole water were collected to act as a comparison with the melting surface ice and to examine how dissolved nutrients were transported through the weathering crust, which is the melting layer of surface ice that has a different physical architecture to the underlying ice (Fig. 4.2). Surface ice habitats were sampled from a 1 × 1 m area chosen at random, from which the top ~ 2 cm of ice was removed using a pre-cleaned ice saw. Samples from all five habitats were collected for the analysis of dissolved inorganic and organic nutrients and dissolved organic carbon (DOC). Algal cell abundances were determined on surface ice samples only. Ice collected for nutrient analysis and algal cell abundance was placed into a sterile Whirl-pak^{TM} bag, while that collected for DOC analysis was transferred into a glass jar that was first rinsed three times with sample. Ice samples were left to melt overnight in the lab tent, typically taking 4-5 h. Supraglacial stream water samples for nutrient

analysis were collected using high-density polyethylene plastic bottles (Nalgene^{TM}), whereas those for DOC analysis were collected in glass jars. Both sampling containers were rinsed three times with sample prior to collection. Cryoconite hole water used for nutrient and DOC analysis was collected using a large pipette and transferred into a Nalgene^{TM} bottle or glass jar, respectively. The large pipette and collection vessels were rinsed three times with sample prior to collection. All high-density polyethylene plastic bottles (Nalgene^{TM}) for nutrient samples were acid washed in ~ 10 % HCl solution prior to first use and all glass jars for DOC samples were furnaced at 500 ºC for four hours prior to first use.

Some 15 ml of the homogenised, unfiltered ice melt and water samples were subsampled and fixed using 25 % glutaraldehyde at 2 % final concentration for quantifying algal cell abundance. These fixed samples were stored outside in the dark at ambient ice sheet temperatures. Ice melt and water samples for nutrient analysis were filtered through a 25 mm, 0.22 µm cellulose nitrate inline syringe filter (Whatman^{TM}) and stored in high density polyethylene plastic bottles (Nalgene^{TM}; 30 mL). The bottles were immediately frozen and stored at a temperature of -20 °C, using a Waeco 32 L Freezer. Ice melt and water samples for DOC analysis were filtered using a glass filtration column and a furnaced 47 mm, 0.7 µm GF/F. The filtration column was washed three times with sample water prior to collection of the filtrate. The filtrate was stored in pre-furnaced amber glass vials and acidified with 100 µL of 1 M HCl. They were chilled to a temperature of ~ 3 °C by storing the samples in a box at ambient air temperature. The samples were maintained at this temperature during transport and in storage at the LowTex Laboratory at the University of Bristol. Nutrient samples were thawed immediately prior to analysis using a ~ 40 °C hot water bath. Procedural blanks (n= *9*) were collected over the course of the sampling season, by processing deionised water in place of sample.

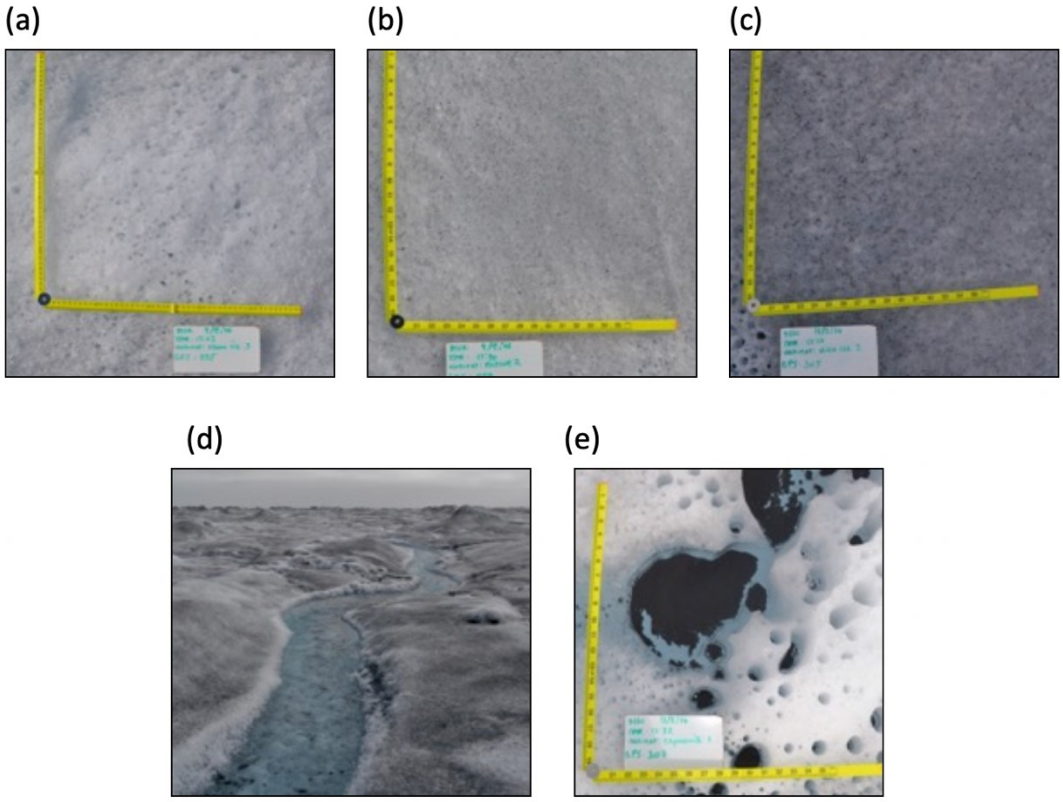

Figure 4.2: The five supraglacial habitats sampled: **(a)** ice with low visible impurities, **(b)** ice with medium visible impurities, **(c)** ice with high visible impurities, **(d)** supraglacial stream, **(e)** cryoconite hole.

4.3.2 Analytical methods

The methods described below are similar to methods described in chapter 2, section 2.3.4.1, yet due to subtle differences (i.e. quantification algal cell abundance and the use of a Lachat QuickChem® 8500 Series 2 Flow Injector Analyzer for dissolved P analysis) the text has been left unabridged.

Algal cell abundance was quantified using a Fuchs-Rosenthal haemocytometer (Lancing, UK) on a Leica DM 2000 epifluorescence microscope with attached MC120 HD microscope camera (Leica, Germany). For samples containing sufficient cell abundance, a minimum of 300 cells were counted to ensure adequate assessment of assemblage diversity (Williamson et al., 2018).

DIN species include NH_4^+, NO_2^- and NO_3^- and were quantified as follows. First, NH_4^+ was quantified spectrophotometrically using a Lachat QuickChem® 8500 Series 2 Flow Injector Analyzer (FIA; QuickChem® Method 31-107-06-1-I). Measurements were based on a phenolate-hypochlorite alkaline reaction method measured at 630 nm (Solorzano, 1969). The limit of detection (LoD) was 0.62 µM, determined by dividing the standard deviation of the response of the calibration curve by the slope of the calibration curve, then multiplying the result by 3 (Shrivastava and Gupta, 2011). Precision was ±2.1 %, and accuracy was +8.5 %, as determined from comparison with a gravimetrically diluted

1000 mg L^{-1} NH_4^+-N certified stock standards to a concentration of 1.1 µM. (Sigma TraceCERT®). Second, NO_2^- and total oxidised nitrogen (TOxN) (NO_2^- + NO_3^-) were quantified spectrophotometrically using a Gallery Plus Automated Photometric Analyzer (Thermo Fisher Scientific, UK). This combination of analysis allows the original NO_3^- concentration to be determined by subtracting NO_2^- from TOxN.

TDN is the sum of DIN and DON, and was determined by digesting the samples with a potassium persulfate, sodium hydroxide and boric acid reagent and autoclaving at 121ºC for 30 minutes (Grasshoff et al., 1999). This process causes the oxidation of organic nitrogen compounds, which can then be measured as TOxN as above. Purification of the potassium persulfate was conducted via recrystallisation in order to remove any N contamination. Measurements were based on the hydrazine-sulfanilamide reaction method measured at 540 nm. DON was then estimated by subtracting DIN from TDN (i.e. DON= TDN-DIN). LoDs were 0.14 µM (NO_2^-), 0.64 µM (TOxN) and 0.87 µM (TDN/DON). Precisions were ±0.87 % (NO_2^-), ±1.17 % (NO_3^-) and ±0.63 % (TDN/DON), and accuracies were -4.04 % (NO_2^-), -8.07 % (NO_3^-) and -5.7 % (TDN/DON), as determined from comparison with gravimetrically diluted 1000 mg L^{-1} NO_2^-N and NO_3^-N certified stock standards to a concentration of 0.71 µM (NO_2^-), 1.4 µM (NO_3^-) and 7.1 µM (TDN/DON) (Sigma TraceCERT®).

TDP (total dissolved phosphorus) is the sum of DIP (principally PO_4^{3-}) and DOP. The same persulfate digestion method described for TDN was used to measure TDP as PO_4^{3-}. PO_4^{3-} in both undigested and the digested samples was quantified using a Lachat QuickChem® 8500 Series 2 Flow Injector Analyzer (FIA; QuickChem® Method 31-115-01-1-I) using the molybdenum blue method measured at 880 nm. DOP was determined by the subtraction of DIP in the undigested sample from the TDP in the digested sample (i.e. DOP = TDP-DIP). The LoD was 0.02 µM (PO_4^{3-} and TDP/DOP). Precisions were ±1.6 % (PO_4^{3-}) and ±3.1 % (TDP/DOP), and accuracies were +2.3 % (PO_4^{3-}) and +5.0 % (TDP/DOP), as determined from comparison with gravimetrically diluted 1000 mg L^{-1} PO_4-P certified stock standards to a concentration of 0.65 µM (Sigma TraceCERT®).

DOC concentrations were quantified using a Shimadzu TOC-L Organic Carbon Analyzer, with a high sensitivity catalyst. Non-purgeable organic carbon (NPOC) was measured after acidification of samples with HCl and catalytic combustion (680 °C) of dissolved organic carbon to carbon dioxide, which was then measured by infrared absorption. The LoD was 9.5 µM. Precision was ±2.4 % and accuracy was -5.9 %, as determined from comparison with gravimetrically diluted 1000 mg L^{-1} TOC certified stock standards to a concentration of 83.3 µM (Sigma TraceCERT®).

4.3.3 Data analysis

All measurements below the LoD were considered to be 0 for all statistical analyses. All DIN, DON, DIP, DOP and DOC data were water blank-corrected using values from the respective field procedural blanks (Table 4.1). Additionally, all blank corrected values that were negative were assumed to be 0 for all statistical analyses. Statistical analysis was performed in RStudio v.1.1.414 (RStudio, Inc 2018). Identification of statistical differences between the nutrient content, DOC concentration and algal cell abundance in different habitats was achieved using 1-way analysis of variance (ANOVA) or t-test comparisons, with post-hoc Tukey HSD analysis applied to all significant ANOVA results. Pearson's product-moment correlations were used to identify correlations between DON, DOC and algal cell abundance. Homogeneity of variance and normality of distribution were tested prior to all parametric analyses, and model assumptions were verified by examination of model criticism plots.

4.4 Results

4.4.1 Dissolved nutrient concentrations in surface ice with differing levels of visible impurities

Supraglacial environments are extremely oligotrophic, making measurements of dissolved nutrients difficult. Dissolved nutrient concentrations reported in previous studies of supraglacial environments are typically below or just above instrument limit of detections. Some 54 DON, 41 DIN, 74 DOP, 40 DIP and 59 DOC samples out of a total of 81 samples for all five supraglacial habitats had concentrations above the LoD.

Dissolved organic concentrations were significantly higher than dissolved inorganic concentrations for nitrogen and phosphorus. Some 93 % of the TDN was in the form of DON and about 67 % of TDP was present in the form of DOP in all three surface ice habitats. Mean DON concentrations for the three surface ice habitats range from 5.1-14.0 μM, while those for DIN range from 0.62-1.0 μM (Fig. 4.3, Table 4.1). Overall, mean DON concentrations for the three ice surface habitats, were significantly higher ($F_{1,71}$=12.4, $p<0.0001$) than mean DIN concentrations. Similarly, DOP concentrations were usually at least twice those of DIP concentrations for the three ice surface habitats, with mean values ranging from 0.10-0.15 μM and 0.03-0.07 μM respectively (Fig. 4.4, Table 4.1). T-tests revealed significant differences between DON and DIN in all three surface ice habitats (low: t_{36}=3.6, $p<0.001$, medium: t_{36}=5.3, $p<0.0001$, high: t_{36}=7.4, $p<0.0001$, (Fig. 4.3) and DOP concentrations as significantly higher than DIP concentrations for all three surface ice habitats (low: t_{36}=3.1, $p<0.01$, medium: t_{36}=2.1, $p<0.05$, high: t_{36}=3.7, $p<0.001$) (Fig. 4.4). DON and DOC concentrations in the three surface ice habitats showed clear trends with increasing visible impurities (Fig. 4.3 & 4.5). DON concentrations increased significantly from low to medium and low to high

visible impurity loadings ($F_{4,71}$=19.8, p<0.05, $F_{4,71}$=19.8, p<0.001, respectively), while DOC concentrations increased significantly in ice with high and low visible impurity loading ($F_{4,74}$=6.8, p<0.01).

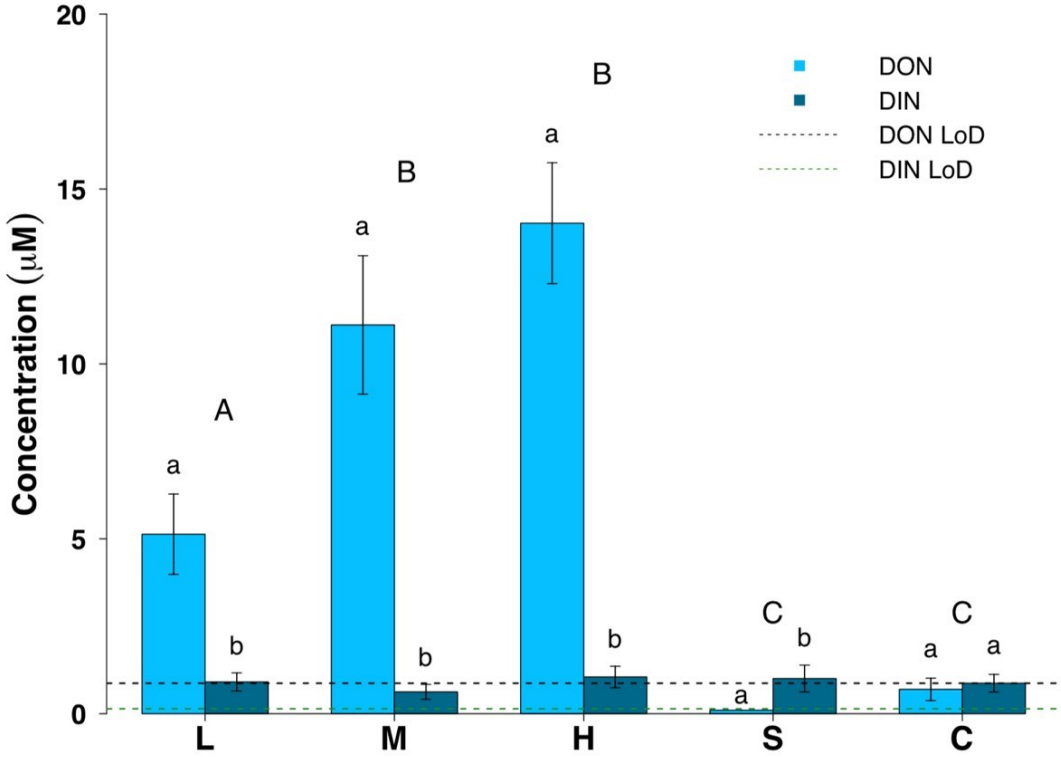

Figure 4.3: Dissolved Organic Nitrogen (DON) and Dissolved Inorganic Nitrogen (DIN) concentrations for all surface habitats (mean ± SE, n=19 for **L**,**M**,**H**, n=10 for **S** and n=14 for **C**). **L**- ice with low visible impurities, **M**- ice with medium visible impurities, **H**- ice with high visible impurities, **S**- supraglacial stream water and **C**- cryoconite hole water. LoD line depicts the limit of detection of the instrument. *Uppercase letters* denote homogeneous subsets derived from post-hoc TukeyHSD analysis on a significant 1-way ANOVA in relation to dissolved nitrogen phase. *Lowercase letters* denote T-test comparisons in relation to habitat type.

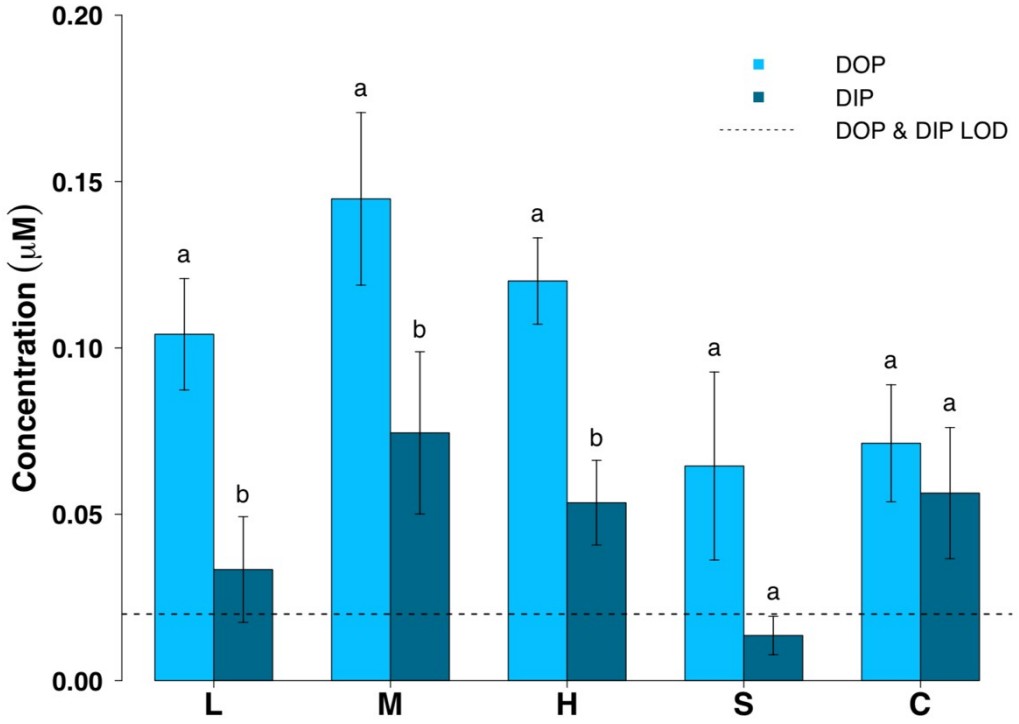

Figure 4.4: Dissolved Organic Phosphorus (DOP) and Dissolved Inorganic Phosphorus (DIP) concentrations for all surface ice habitats (mean ± SE, n=19 for **L**,**M**,**H**, n=10 for **S** and n=14 for **C**). **L**- ice with low visible impurities, **M**- ice with medium visible impurities, **H**- ice with high visible impurities, **S**- supraglacial stream water and **C**- cryoconite hole water. LoD line depicts the limit of detection of the instrument. *Lowercase letters* denote T-test comparisons in relation to habitat type.

4.4.2 Links between algal abundance and dissolved organic nutrients

Algal cell abundance, which ranged from 90 cells ml^{-1} to 0.98 × 10^4 cells ml^{-1}, increased significantly with the amount of visible impurities seen on the ice surface, as shown in Figure 4.6 ($F_{2,54}$=26.1, $p<0.0001$). No determination of the mineralogic composition of the visible impurities was conducted. A Pearson's product-moment correlation was undertaken to illustrate the relationship between average algal abundance and average DOC and DON concentrations, as DOC and DON concentrations also increased with the amount of visible impurities present. Correlations between average algal cell counts versus both DON and DOC surface ice concentrations were significant (t_3=3.5, $p<0.05$, r=0.9 and t_3=5.4, $p<0.01$, r=0.95, respectively). Comparison of DOP surface ice concentrations and algal cell counts were not significant.

Dissolved organic nutrient ratios were assessed to investigate the presence of a limiting nutrient. Molar DON:DOP ratios, ranging from 49 to 120, were elevated for all three surface ice environments compared to the 16:1 Redfield Ratio, and DOC:DOP ratios for all three surface ice habitats, which ranged from 800 to 2000, were considerably higher, as much as ~ 19 times the Redfield ratio, 106:1

(Table 4.1). DOC:DON ratios, which ranged from 16 to 17, were, on average, twice the balanced 6.6:1 ratio (Table 4.1). DON:DOP and DOC:DOP ratios also increased with the amount of visible impurities present, while DOC:DON ratios remain relatively constant for the three surface ice habitats (Table 4.1).

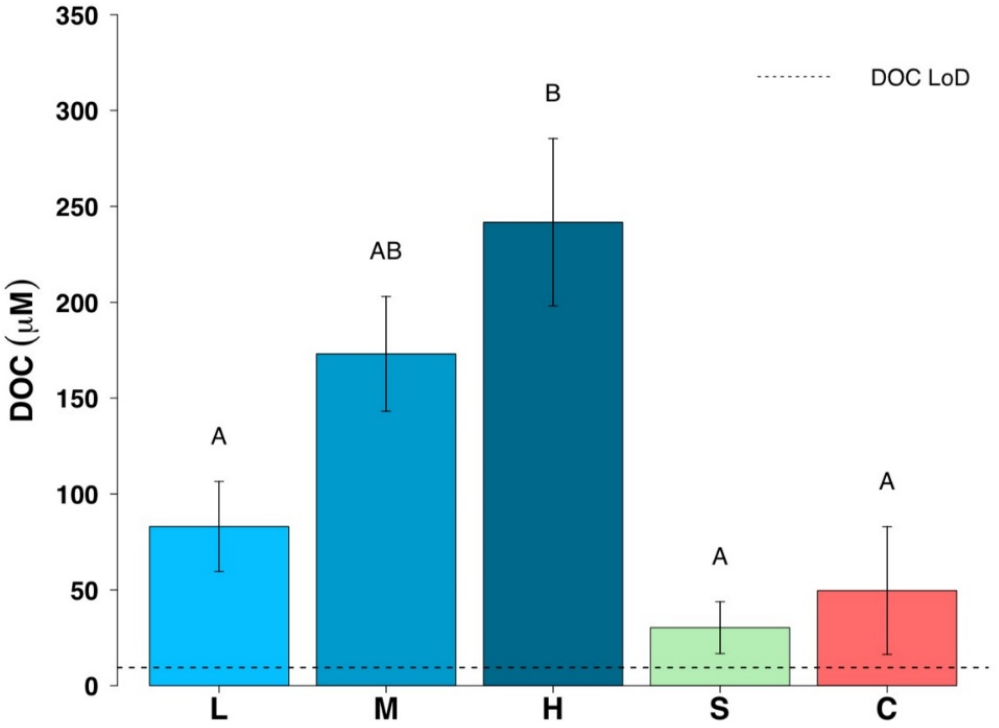

Figure 4.5: Dissolved Organic Carbon (DOC) concentrations for all five surface habitats (mean ± SE, n=19 for **L**,**M**,**H**, n=10 for **S** and n=14 for **C**). **L**- ice with low visible impurities, **M**- ice with medium visible impurities, **H**- ice with high visible impurities, **S**- supraglacial stream water and **C**- cryoconite hole water. LoD line depicts the limit of detection of the instrument. *Uppercase letters* denote homogeneous subsets derived from post-hoc TukeyHSD analysis on a significant 1-way ANOVA in relation to habitat type.

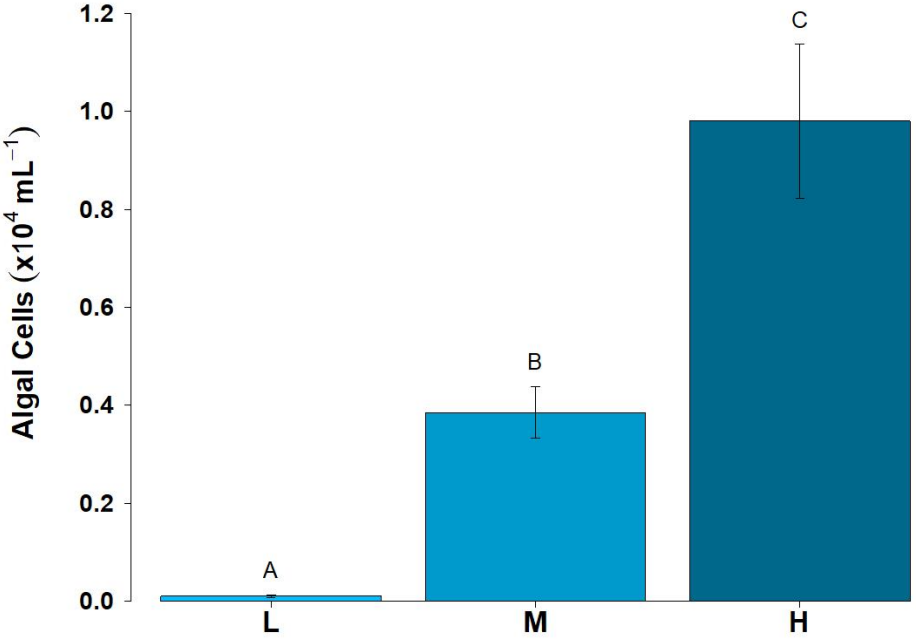

Figure 4.6: Algal cell abundance in ice surface ice habitats (mean ± SE, n=19 for each habitat). **L**- ice with low visible impurities, **M**- ice with medium visible impurities and **H**- ice with high visible impurities. *Uppercase letters* denote homogeneous subsets derived from post-hoc TukeyHSD analysis on a significant 1-way ANOVA in relation to habitat type.

4.4.3 Low transport of dissolved organic nutrients within the water table

Mean DON and DOP concentrations were significantly lower in supraglacial streams (0 µM and 0.07 µM, respectively) and cryoconite hole water (0.7 µM and 0.07 µM, respectively) compared to low, medium and high visible impurity ice. All DON concentrations for cryoconite hole and supraglacial stream water were below the LoD (Fig. 4.3). DIN concentrations were relatively constant over all supraglacial habitats with mean concentrations ranging from 0.62 µM to 1.0 µM. DOP concentrations in supraglacial stream (0.07 ± 0.03 µM) and cryoconite hole water (0.07 ± 0.02 µM µM) were not significantly different from DIP concentrations (0.01 ± 0.01 µM and 0.06 ± 0.02 µM, respectively). DIP concentrations in low (0.03 ± 0.02 µM), medium (0.07 ± 0.02 µM) and high (0.05 ± 0.01 µM) visible impurity ice were only slightly elevated compared to supraglacial streams, whereas cryoconite hole water concentrations were comparable to the three surface ice habitats. Mean DOC concentrations in supraglacial stream and cryoconite hole water (30 µM and 50 µM, respectively) were significantly lower than ice with high visible impurities ($F_{4,74}$=6.8, $p<0.001$, in both cases) (Fig. 4.5).

Table 4.1: Summary statistics for dissolved macronutrient (N and P) and DOC concentrations in the five supraglacial habitats. DON, DIP, DOP and DOC denote Dissolved Organic Nitrogen, Dissolved Inorganic Phosphorus, Dissolved Organic Phosphorus and Dissolved Organic Carbon respectively.

	Ice Habitat			**Supraglacial Stream**	**Cryoconite Hole**	**Field Blank**
	Low	**Medium**	**High**			
NH_4^+	0.91±0.26 0-3.8	0.62±0.21 0-2.9	1.0±0.31 0-4.3	1.0±0.38 0-3.1	0.87±0.25 0-2.7	0.80±0.32 0-2.6
NO_2^-	0.00±0.00 0	0.00±0.00 0	0.00±0.00 0	0.00±0.00 0	0.00±0.00 0	0.00±0.00 0
NO_3^-	0.00±0.00 0	0.00±0.00 0	0.00±0.00 0	0.00±0.00 0	0.00±0.00 0	0.00±0.00 0
DON	5.1±1.1 0-10	11±2.0 0-40	14±1.7 3.2-27	0.00±0.00 0-0.82	0.70±0.32 0-3.2	0±0 0
DIP	0.03±0.02 0-0.27	0.07±0.02 0-0.44	0.05±0.01 0-0.20	0.01±0.01 0-0.04	0.06±0.02 0-0.23	0.00±0.00 0
DOP	0.10±0.02 0-0.27	0.15±0.02 0-0.48	0.12±0.01 0-0.25	0.07±0.03 0-29	0.07±0.02 0-0.22	0.00±0.00 0-0.04
DOC	83±24 0-350	173±30 29-451	242±44 0-636	30±13 0-84	50±33 0-435	12±7.7 0-35
DON:DOP	49.3	78.9	116.8	0.00	9.4	Na
DOC:DOP	797.8	1166.2	2013.3	455.3	671.3	Na
DOC:DON	16.2	15.6	17.2	Na	71.3	Na
DIN:DIP	27.2	8.4	19.6	74.1	15.5	Na
Sample Size (n)	19	19	19	10	14	9

For each nutrient, the mean ± SD is provided, followed by the range of values. Concentrations are expressed in μM; nutrient ratios are in μM/μM.

4.5 Discussion

4.5.1 Dominance of dissolved organic over dissolved inorganic phases in melting ice surface environments

Dissolved organic nutrients (DON and DOP) dominate dissolved inorganic nutrients (DIN and DIP) in the melting surface ice environments of this region of the Dark Zone (Fig. 4.3 & 4.4), in contrast with the dominance of DIN in studies of fresh snow or ice cores from the GrIS (Telling et al., 2012b; Wolff, 2013), which has a mean concentration of 1.4 μM. Further, DIN also dominates on the margins of the ice sheet, where Telling et al., (2012) found DIN concentrations in snow to be 1.4±0.2 mM, but DON concentrations to be non-detectable. Wadham et al., (2016) reported relatively similar DIN (1.3 μM) and DON (~ 1.6 μM, assuming DON = TDN-DIN in their tabulated data)

concentrations in a small number (n = 7) surface, debris-rich ice in the Dark Zone of the GrIS during the main ablation season, but these values were thought likely to be associated with dispersed cryoconite, the dark organic-rich sediment that accumulates in the bottom of cryoconite holes and larger supraglacial water bodies. Otherwise, DON was not measurable in snow and surface ice, prior to melting. In summary, this suggests that potential input of dissolved N-species to supraglacial environments from fresh snow and melting meteoric ice are dominated by DIN, rather than DON. There is too little data on DIP and DOP to be confident that the this is also the case for P species. By contrast, dissolved N-species in the melting ice surface of the Dark Zone shifts to a domination of DON during the peak ablation season (July and August), when blooming of glacier algae occurs. We therefore hypothesise that the algae present in these blooms drive the shift in nutrients during the peak ablation season from the dissolved inorganic phase to the dissolved organic phase.

4.5.2 Association of dissolved organic nutrients and algal abundance

Figure 4.6 shows that algal abundance increases in the ice with low, medium and high visible impurities. The blooming of the algal cells is also associated with trapping of other mineral particulates at the surface. Yallop et al., (2012) reported a 3:2 mineral particle to algal cell ratio for surface ice collected in the Dark Zone, although these particles have only a minor impact on the albedo reduction at the surface (Cook et al., 2019). It is clear from Fig. 4.3 that the mean DON concentration increases from low to high visible impurities, consistent with DON formation being linked to glacier algae blooms. This is most likely due to a combination of extracellular exudation of polymeric substances and the decomposition of glacier algal cells within the supraglacial system. Concentrations of NO_3^- and NO_2^- are zero (Table 1), and NH_4^+ is the only measurable DIN species (mean values range from 0.6 to 1 μM). The absence of measurable NO_3^- and NO_2^- is consistent with the uptake of these species by glacier algae, and the emergence of NH_4^+ as the dominant DIN species is consistent with heterotrophic remineralization of organic matter (Telling et al., 2012b). We note that the mass of N held in the microbial biomass is likely increasing over time, since the sum of the mean DIN and DON concentrations (1.0 μM and 0.0 μM respectively) in the supraglacial stream water, which is the ultimate sink of macronutrients from the melting ice surface, is less than the average DIN concentration of the melting ice (1.4 μM) (Telling et al., 2012b; Wolff, 2013; Wadham et al., 2016). The only measurable DIN species in supraglacial meltwater is NH_4^+, which points to ammonification being an important process in terms of N dynamics and loss of labile N from the melting surface ice. Previous studies of the relative rates of primary production and bacterial production in both the margins and the Dark Zone have produced ratios of 30:1 (Yallop et al., 2012; Nicholes et al., 2019). The dominance of dissolved organic nutrients and NH_4^+ in surface ice environments documented here, in combination with reduced secondary production relative to net

primary production in the same environments, indicates an inefficiency in the microbial loop for remineralization of organic nutrient N-stocks (Fig. 4.7).

There is less data in the literature on the relative abundance of DIP and DOP in snow and ice, but here we show that there are similarities between the accumulation of dissolved N into DON and P into DOP species (Fig. 4.3 & 4.4). Mean DOP concentrations in the five sampled environments are higher than mean DIP. This is consistent with uptake of P by glacier algae and subsequent recycling of P into organic forms. An interesting observation is that there is usually some measurable DIP found in the particulate-rich environments that were sampled (surface ice and cryoconite hole water), whereas the mean DIP in the supraglacial stream water is the lowest value recorded and below the limit of detection. This suggests two things. First, that particulates are the source of DIP, and second, that export of P from the melting surface ice is largely as DOP. We noted above that particulates are associated with glacier algae in the melting surface ice, and it appears that as algal blooms develop, more particulates become trapped in the surface layer (Yallop et al., 2012). It may well be that there is an "inorganic symbiosis" between the glacier algae and the trapped particulates, which provide a P source for algal growth.

Our results on the dominance of DON and DOP are consistent with findings of previous studies in polar glacier surface aquatic environments (Stibal et al., 2008a; Stibal et al., 2008b; Stibal et al., 2009; Wadham et al., 2016). For example, Stibal et al., (2008) reported that DON (~ 72 %) and DOP (~ 89 %) in waters in cryoconite holes on a Svalbard glacier dominated the total dissolved N and P pools. Wadham et al., (2016) found elevated DON concentrations in water in cryoconite holes and debris-rich surface ice in the Dark Zone, suggesting either mineralization of organic matter by microbial activity or leaching of allochthonous organic matter in debris. These observations suggest that conversion of dissolved inorganic to dissolved organic nutrients by microbial communities in melting surface ice environments may be a common process on glacier surfaces

4.5.3 Retention of nutrients at ice sheet surface

The low concentration of DIN, DIP, DON and DOP in the supraglacial meltwaters relative to the melting surface ice suggests that macronutrients are retained in these surface environments. Melting ice surfaces in the Dark Zone often have a veneer of low density, wet porous ice, which may reach depths of 1-2 m, known as the "weathering crust" (LaChapelle, 1959; Müller and Keeler, 1969; Munro, 1990; Irvine-Fynn et al., 2012). The intense short wave radiation during summer often causes internal melt along ice crystal boundaries, resulting in a surface ice layer with heterogeneous thickness, density, porosity and water content (Müller and Keeler, 1969; Cook et al., 2016a; Christner et al., 2018). The porous nature of the weathering crust allows flow paths to form through the water

table that exists within the surface ice (Irvine-Fynn et al., 2012; Cook et al., 2016a; Rassner et al., 2016; Christner et al., 2018), which act as important links between different supraglacial environments and are believed to transport microbes and nutrients via subsurface flow (Irvine-Fynn et al., 2012; Hoffman et al., 2014; Karlstrom et al., 2014; Cook et al., 2016a). Water is often in temporary storage in the weathering crust (Irvine-Fynn et al., 2012), particularly at depth where connectivity of flow paths can be low. It follows that the first explanation for retention of dissolved organic nutrients in the weathering crust is that they accumulate in water stored in the weathering crust.

DOC concentrations in supraglacial stream water were lower than the DOC in all surface ice habitats, particularly surface ice with high visible impurities (Fig. 4.5). This suggests a second possible mechanism of retention of DON and DOP in the weathering crust, via the production of extracellular polymeric substances (EPS). Algae and bacteria produce EPS which can alter the physical and chemical environment around their cells (Stibal et al., 2012b; Angelaalincy et al., 2017). For example, it has been shown that EPS are used by cyanobacteria in cryoconite holes to bind mineral particles together creating the cryoconite granules at the bottom of the hole (Stibal et al., 2012a; Yallop et al., 2012; Musilova et al., 2016). EPS is often colliodal (here, operationally defined as passing through 0.4 μm, but not 0.02 μm filter membranes) (Raiswell et al., 2018), and when analysed from filtered (through 0.4 μm membranes), melted surface ice samples will be in the dissolved organic fraction (Pereira et al., 2009; Hodson et al., 2010b). The chemical composition of EPS exuded by glacier algae is unknown. We note that the EPS of bacteria living in sewage sludge can have a molar C:N:P ratios that approaches 100:101:14 (Guibaud et al., 2008), in order to illustrate that EPS can contain N and P. It is likely that the EPS of glacier algae contains relatively more C than N and P, given the depauperate nature of the melting ice surface. EPS certainly seem to be associated with the binding and retention of particulates in the weatheirng crust, and it follows at least some of the DON and DOP may also be associated with this EPS.

These two mechanisms of retention of dissolved organic nutrient in the weathering crust, either in temporarily stored water or as EPS, mean that DOC, DON and DOP storage in the weathering crust is transitory, and given the dynamic response of the weathering crust to climatic perturbations, it is very likely that export of these species from the weathering crust will be pulsed, rather than constant. For example, large melt events, accompanying summer storms, may result in wholescale melting of the weathering crust (Tedstone et al., In Review), and export of a significant quantities of the dissolved organic phases contained within them. By contrast, that stored in the weathering crust towards the end of the ablation season, when the crust is freezing and water flow paths are closing, may be retained in the frozen ice surface overwinter. For example, Musilova et al., (2017) reported

that at the margin of the GrIS, DOC remaining in surface ice at the end of the ablation season likely froze over winter and was released the following ablation season through ice melt.

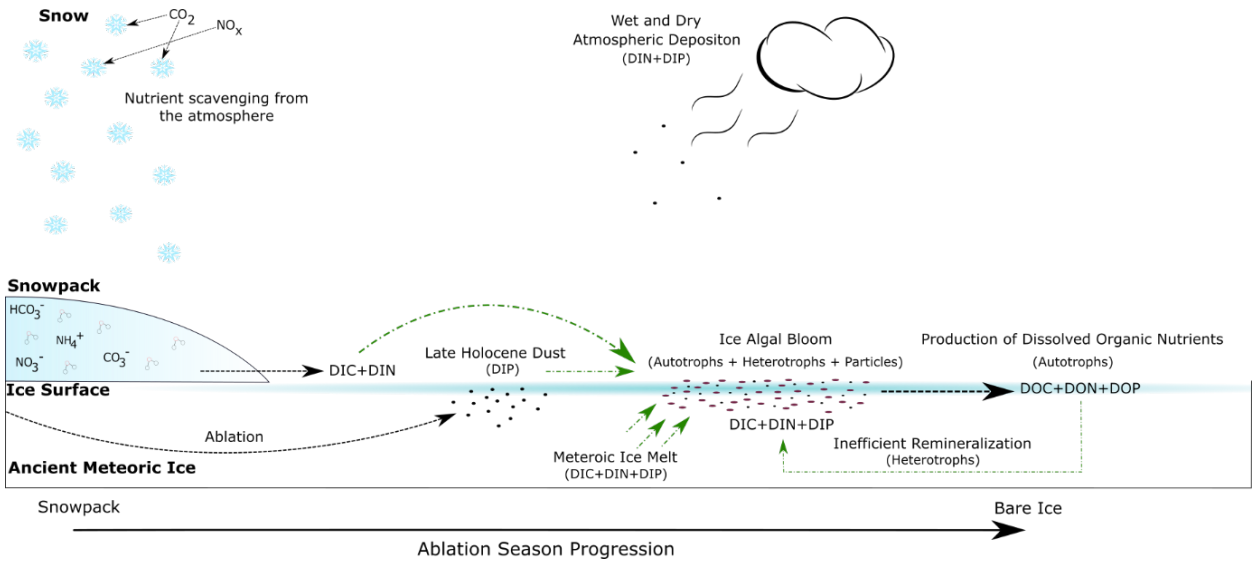

Figure 4.7: Conceptual diagram of the supraglacial environment in the Dark Zone of the GrIS. Black dashed lines represent nutrient inputs to all supraglacial environments. Green lines represent hypothesized nutrient inputs utilized by ice algal blooms. Arrow thickness represents relative nutrient concentration.

4.5.4 Stoichiometry of different supraglacial environments

DOC:DON:DOP ratios in melted surface ice samples may provide information on whether N or P is the limiting nutrient within supraglacial environments in the Dark Zone. For example, Table 1 shows that DON:DOP ratios increase systematically, from 49, 78 to 120, for low, medium and high impurity surface ice environments respectively, as do DOC:DOP ratios (800, 1200, 2000). By contrast, DOC:DON ratios remain relatively stable for the surface ice habitats (16, 16 and 17, respectively). This could indicate that P is limiting for the glacier algal community. However, this does not quite tie in with the DIP data presented in Fig. 4.4, which shows that measurable, if low, concentrations of P are usually present in the melting surface ice. Rather, NO_3^- and NO_2^- are below detection, presumably as a result of uptake by phototrophs, and NH_4^+ is the only measurable DIN species, presumably as a result of heterotrophic activity. Phototrophs preferentially utilize both NH_4^+ and NO_3^-, and the presence of both DIN and DIP in melting surface ice environments, irrespective of visible particulate loading, and therefore of algal cell abundance, suggests that a factor other than macronutrient concentration is limiting algal growth. Table 4.1 shows that mean NH_4^+ concentrations in the melting surface ice are in the range of 0.6 – 1.0 µM. We noted above that there is no readily available C:N ratio of glacier algae in the literature, but typical C:N ratios of sea ice algae are in the range of 12-46 (Niemi and Michel, 2015). This implies that somewhere in the range of 7.2 – 46 µM of C could be additionally fixed, if all the N was taken up by phototrophs with this range of C:N ratios. We also

noted that it is even more difficult to find C:N:P ratios of glacier algae, but should the C:P ratio be in the region of 1000:1 to 100:1, then P demand will be 0.007 – 0.46 μM. Table 4.1 shows that the mean concentration of DIP in melting surface ice is in the range of 0.03 to 0.05 μM, which suggests that P is not a limiting macronutrient on primary production. The systematic change in DON:DOP and DOC:DOP ratios with increasing visible impurities, a proxy for algal cell abundance, could be driven by the amount of P per cell that is potentially available at the high light intensity of the ablation season (> 1500 μmol photons m^2 s^{-1}). The DIP content of the surface ice is relatively constant (Table 4.1) given the much larger change in cell abundance as the visible impurities increase. The combination of lower P availability at high light intensity results in an increase in the C:P ratio of phototrophs in other aquatic environments (Hessen et al., 2013). It is plausible that this too happens with glacier algae, and that subsequent decomposition products and EPS will likewise have higher DOC:DOP ratios as a consequence.

4.6 Conclusion

We conclude that DIN and DON concentrations in the melting surface ice of the Dark Zone on the GrIS are markedly different from those documented in ice cores to date. Wolff et al., 2013 reported DIN, principally in the form of NO_3^-, dominating the initial composition of ice melt, yet in the present study, DON dominates the melting surface ice environments which host blooming glacier algae. Furthermore, DIN in these environments is exclusively present as NH_4^+, and NO_3^- is below the detection limit (0.64 μM). There is relatively little data on the P content of Greenland ice, but we find that DOP dominates DIP in melting surface ice habitats, although DIP is usually present in measurable quantities (LoD = 0.02 μM). The presence of both NH_4^+ and DIP, even in heavily colonised melting surface ice, suggests that factors other than macronutrient limitation control the blooms. We speculate that dissolved macronutrients are held in the melting surface ice because of the architecture of the weathering crust, and/or because EPS is retained within the melting ice latticework. The former controls the hydrology and the connectivity of water flow paths and water storage in the surface ice, and the latter may be involved with the retention of particulates in the surface. There is currently no data on C:N:P ratios of EPS exuded by glacier algae, but EPS of other autotrophs does contain both N and P in association with C. DOC:DON ratios are relatively constant in melting surface ice, but DOC:DOP ratios increase markedly with increasing algal cell counts. This may be attributable to the increasing cell to DIP ratio, which, at high light intensity, increases the C:P ratio of autotrophs in other freshwater environments (Hessen et al., 2013). This could be seen as a beneficial adaption to algal life in melting ice surfaces, where P sources are limited, since blooms are not so dependent on P as a consequence.

Chapter 5: Concluding Discussion

The primary aim of this research project was to better understand nutrient input and abundance in supraglacial environments, linking their change to meteorological or biological factors. The focus has been on dissolved macronutrients (N and P) as well as dissolved organic carbon due to their roles in supporting primary and secondary production in snow pack and surface ice environments. Biogeochemical cycling in bare ice and snow pack habitats has received little attention in the literature compared to cryoconite hole habitats, yet in recent studies both have proved to host abundant and active autotrophic and heterotrophic communities capable of significantly impacting global nutrient and carbon cycles. This final chapter summarises the main findings of this thesis, as well as provides a synthesis of the overall findings. It concludes by addressing the limitations of this thesis, persisting knowledge gaps and directions for future research.

5.1 Summary of main findings

5.1.1 Snow pack heterotrophic communities respond to nutrient additions under simulated polar winter conditions

The research presented in Chapter 2 endeavoured to address the current limited knowledge of microbial activity in snow pack environments during polar winter, as well as the potential for inorganic glacial particles to act as a source of P to the heterotrophic environment. Under simulated polar winter conditions, -5 °C and 24-hour darkness, a 5-week laboratory incubation was conducted allowing for a temporal analysis of heterotrophic community and abundance change in response to nutrient additions. Particulate phosphorus analysis was also performed to investigate microbial utilization of particles as a phosphorus source. This is only the second study to analyse heterotrophic activity in a snow pack environment under polar winter conditions, a season which has previously been considered inert, and the only study to investigate an association between snow pack heterotrophs and a particulate nutrient source. The results demonstrate a heterotrophic community response to different nutrient additions in a cold, dark snow pack as well as provides evidence for utilization of particulate phosphorus by early colonizing bacteria.

1a – Nutrient dependent heterotrophic community response

Elevated bacterial abundance in the DIN addition treatment over time, coupled with the decrease in genera richness, suggests a potential specialization of the heterotrophic bacteria in a N rich environment. The presence of bacteria associated with the nitrogen cycle throughout the time series, e.g. *Bacillus* and *Caulobacter*, and the emergence of others, e.g. *Nitrospira*, by the final time point further supports this. Conversely, in a P rich environment, the microbial community as a whole

appears to benefit and diversification occurs, as seen by an elevated abundance in the final time point of the DIP addition treatment, as well as a significant increase in genera richness. As sporadic and concentrated N inputs via wet and dry deposition are common during the polar night, the specialization seen in this experiment likely occurs *in-situ*, potentially leading to enhanced microbially mediated N cycling.

1b – Colonization of particles by early colonizing bacteria present in snow pack environments

Both bacterial abundance and genera richness were elevated in the particle addition treatment compared to the untreated, furnaced particle control, suggesting a potential colonization of the particles by the snow pack heterotrophic community. A further increase in both bacterial abundance and genera richness in the particle addition treatment over time, indicates increased colonization over the 5-weeks. 16S sequencing revealed only certain genera from the snow pack environment were capable of associating with the particles, as only 73 genera were shared between the snow control and the particles from the particle addition treatment. *Betaproteobacteria*, a class previously identified as early colonizing bacteria, comprised several genera of the mutual 73, with one genus even linked to solubilizing phosphate (Zheng et al., 2017).

1c – Potential utilization of particulate phosphorus by early colonizing heterotrophic bacteria

An inverse relationship between particulate inorganic phosphorus and dissolved inorganic phosphorus indicates a rebinding of dissolved phosphorus onto available particulate receptor sites, creating new, loosely bound particulate inorganic phosphorus accessible to microbial communities (Hodson et al., 2004; Stibal et al., 2008b). Extract 1 from the particulate phosphorus results comprised the lowest concentration of overall particulate inorganic phosphorus, suggesting it was highly accessed by the microbial communities. Changes in microbial biomass phosphorus indicate an initial utilization of internal phosphorus stores, before accessing the newly adsorbed, bioavailable phosphorus, a method found to be applied by many other heterotrophic communities (Kornberg, 1995; White et al., 2010; Achbergerová and Nahálka, 2011; Tocheva et al., 2013). If snow pack heterotrophic communities can access particulate inorganic phosphorus, it may account for the lack of phosphorus limitation observed.

5.1.2 Snow and ice melt are important nutrient sources to developing microbial communities within and beneath a decaying snow pack during the spring thaw

The research presented in Chapter 3 endeavoured to address the current limited knowledge of nutrient inputs to supraglacial environments via snow and ice melt during the spring thaw. During a spring field campaign (June – July) in the GrIS Dark Zone samples of the snow pack, snowmelt, snow covered surface ice and shallow meteoric ice were collected to produce the first preliminary data set

documenting nutrients released from the snow pack and ablating ice surface during the early ablation season. MAR, a regional climate model, was also used to calculate parameters we were unable to measure *in-situ*, such as melt water production and subsurface ice temperature. At present, this is the most comprehensive dataset of nutrient inputs to bare ice habitats and one of the first to present evidence for the initiation and development of microbial communities in the ice surface before the complete ablation of the snow pack.

2a – Snow pack meltwater releases constant nitrate while potential microbial activity influence ammonium and dissolved organic nitrogen levels

NO_3^- concentrations released from the snow pack remained relatively consistent over the sampling period and followed major ion concentration changes, to a first approximation, suggesting that NO_3^- released from the snow pack in the GrIS Dark Zone is associated with factors such as melt rate variation and solute banding. However, NH_4^+ and DON concentrations fluctuated greatly in snow pack meltwater, occasionally surpassing their respective average snow pack concentrations, while also resulting below detection limits on other days. The variations in NH_4^+ and DON in snow pack meltwater could indicate active microbial communities in the snow pack environment as both NH_4^+ and DON concentrations are commonly influenced by microbial activity.

2b – Potential microbial activity and nutrient scavenging from ablating snow covered surface ice at snow-ice interface

Flooded snow pits allowed for insight into nutrient abundance flowing through the snow pack and at the snow-ice interface. Elevated concentrations of both NO_3^- and DON in the flooded snow pit meltwater compared to concentrations in the snow pack suggest that nutrients were scavenged from the surface and shallow meteoric ice by this pooled meltwater, as NO_3^- and DON concentrations in the ice were higher than in the snow pack. Conversely, NH_4^+ concentrations in the flooded snow pit meltwater were mostly below detection even though concentrations in the ice were high, suggesting potential microbial assimilation at the snow-ice interface.

2c – Potential formation of microenvironments in surface ice under decaying snow pack capable of hosting metabolizing glacier algae

MAR, a regional climate model, calcluated a temperature of 0 °C for the top ~ 10 cm of ice underlying a decaying snow pack in the GrIS Dark Zone in June 2017, likely caused by the formation of superimposed ice and the absorption of solar radiation penetrating through the shallow snow pack. The absorption of solar radiation was likely enhanced by dark impurities, including glacier algae cells frozen into the ice from the previous year algal bloom, resulting in localized meltwater and the formation of micro-environments rich in snow and ice meltwater nutrients, potentially able to host

metabolizing glacier algae before the complete ablation of the snow pack. Calculations from MAR were verified by field observations and measurements such as snowpack ablation rate, periods of high meltwater production, snowpack depth and precipitations events.

5.1.3 Surface ice dominated by glacier algae blooms are areas of high dissolved organic nutrient and carbon accumulation

The research presented in Chapter 4 endeavoured to address the current lack of knowledge regarding nutrient and organic carbon abundance and dynamics in bare ice habitats inhabited by glacier algae. By collecting *in-situ* samples of surface ice with differing levels of visible surface impurities and quantifying dissolved nutrients, both inorganic and organic, as well as organic carbon, the first comprehensive data set on nutrient abundances in surface ice hosting a glacier algal bloom was produced. Supraglacial streams, cryoconite hole water and algal cell abundances were also measured to aid data interpretation regarding differences observed between differing levels of visible surface impurities as well as potential transport to downstream environments. To date, this is the most thorough and detailed report of nutrient dynamics in bare ice habitats dominated by glacier algae. Moreover, it is one of the few studies to not only measure dissolved organic nutrients but show they cannot be ignored when considering supraglacial environments.

3a – Dissolved organic nitrogen and phosphorus are the principle phases of N and P in surface ice hosting glacier algae blooms

In surface ice collected from the Dark Zone of the GrIS, ~ 93 % of the total dissolved N was in the form of DON, while ~ 67 % of the total dissolved P was in the dissolved organic phase. DON and DOC increased significantly with increasing visible surface impurities, as did algal cell counts, strongly indicating the dissolved organic fraction quantified was algal derived. DIN concentrations, comprised predominately of ammonium, were low in surface ice habitats, resulting just above detection limits. As ammonium is a common biproduct of organic matter remineralization (Telling et al., 2012b), and low rates of bacterial production have previously been reported for surface ice inhabited by glacier algae (Yallop et al., 2012; Nicholes et al., 2019), we suggest the presence of an inefficient microbial loop for remineralising the dissolve organic pool.

3b – Measurable ammonium and dissolved inorganic phosphorus suggest that P may not be the limiting nutrient it was previously considered

DIN was quantified just above or at the detection limit for all surface ice samples, whereas DIP resulted well above detection limits. Moreover, ammonium comprised the majority of the DIN pool, as described above, while nitrate and nitrite concentrations were mostly undetectable. While

ammonium is commonly a production of organic matter remineralization (Telling et al., 2012b), ammonium photoproduction from dissolved organic matter has also been found in aquatic environments (Bushaw et al., 1996; Wang et al., 2000), suggesting that these two processes may have assisted in slowly restoring the depleted DIN stock. As chemical and physical weathering of particles in the surface ice may have elevated DIP stocks, at present, there are no investigations reporting this process for surface ice hosting glacier algae blooms, thus strongly indicating DIN was depleted at a higher rate than DIP, contradicting the current theory of P limitation in supraglacial environments (Stibal et al., 2008b; Stibal et al., 2009). Factors other than nutrient abundance may also be limiting glacier algae blooms, such as high levels of UV radiation, time since snow line retreat or even the slope of the ice, although at present, knowledge regarding the effects these other factors is also lacking.

3c – Glacier algae blooms display a retention ability at ice sheet surface

Low dissolved nutrient abundance quantified in supraglacial meltwaters, i.e. supraglacial streams and cryoconite hole water, compared to the surface ice suggests a retention of nutrients in the ice surface, for which we suggested two possible methods. First, dissolved nutrients are held at the ice surface through storage in the weathering crust, the top 1 – 2 m of the ice surface, which has been found to act as a porous media where flow paths can form between supraglacial environments (Irvine-Fynn et al., 2012; Hoffman et al., 2014; Karlstrom et al., 2014), transporting nutrients and microbes. Second, the concurrent low abundance of DOC in supraglacial meltwaters suggests the retention of DON and DOP at the ice surface through the production of extra polymeric substances, commonly produced by algae and bacteria (Stibal et al., 2012a; Angelaalincy et al., 2017). This possible retention may allow for furthered recycling and utilization of dissolved organic nutrients by surface ice microbial communities.

5.2 Synthesis

The overarching goal of this thesis was to further the current knowledge regarding nutrient input and abundance in supraglacial environments and link their changes to biological or meteorological factors. As such, the research presented here endeavours to provide a comprehensive body of work which encompasses nutrient dynamics during the polar winter, followed by the spring thaw and finally the glacier algae bloom itself. The results provide evidence for microbially mediated nutrient cycling not only during the main growth season of late spring and summer, but also during both winter and early spring. This understanding is vital as microbial activity during the polar winter will likely have implications for nutrients released during the spring thaw, which stimulate the initiation and growth of the algal bloom. It is important to note that evidence of microbially mediated nutrient cycling during

the polar winter occurred in snow sampled from Svalbard, which has different nutrient concentrations, wet and dry deposition events and anthropogenic influences compared to Greenland. Yet, the heterotrophic communities detected in the snow used for the incubation are commonly found throughout the Arctic (Lutz et al., 2016), suggesting that the results shown in this experiment are likely comparable to Greenland. These finding also show that bare ice habitats hosting large scale algal blooms are areas of high dissolved organic C, N and P accumulation, previously unreported in the literature. Supraglacial environments are well documented as a key nutrient source to englacial, subglacial and downstream environments, as such, bare ice habitats hosting abundant algal blooms may be a significant source of dissolved organic macronutrients to these downstream environments. Subglacial environments are commonly dominated by chemoheterotrophs and chemoorganotrophs, which have been found to aid the dissolution of nutrients from ice and bedrock leading to increased nutrient export (Skidmore et al., 2000; Tranter et al., 2002; Tranter et al., 2005; Hodson et al., 2008). Thus, labile, dissolved organic nutrients transported from surface ice habitats may stimulate microbial activity in subglacial environments and potentially increase nutrient export further. This will likely have knock on effects for costal environment as direct evidence has now been found for glacial stimulation of coastal productivity (Wadham et al., 2019). Further, some dissolved organic nutrients frozen into the ice surface at the onset of winter were also found in ablating surface ice during the following spring thaw, thus also providing a nutrient source for the following season algal bloom. These advancements in the current knowledge of nutrient cycling and microbial communities in supraglacial environments is critical for areas such as the GrIS Dark Zone, a study area that was the focus of this thesis, as the blooms of glacier algae present in this stretch of bare ice extending along the south-west coast have been shown to significantly influence surface albedo decline and consequently increased melt rates of the GrIS. As the GrIS is one of the largest contributors to global sea level rise, the extent, magnitude and duration of these algal blooms holds great importance for future melt rate predictions and sea level rise.

5.3 Limitations and future work

While several significant findings have been made throughout this thesis, there remains much to understand regarding nutrient abundance, input and cycling in supraglacial environments. The limitations of this PhD and areas for future work are outlined below.

- ***In-situ* sampling conducted was constrained to only one small sampling area of the Dark Zone on the GrIS.** The Dark Zone extends roughly along the entire south-west coast of the GrIS, yet sampling was only conducted along the K-transect near S6 weather station, a common transect and sampling area of the Dark Zone, for both field seasons due to field logistic constraints. More expansive sampling of the Dark Zone, other areas of the GrIS and

the cryosphere, where glacier algae have been reported, needs to be conducted for comparison and upscaling purposes. The results from this PhD provide a strong preliminary and exploratory data set, but confirmation of these results from other areas is needed.

- **Lack of quantification of microbially mediated nutrient cycling processes.** Due to the limited literature regarding nutrient abundance and input to supraglacial environments, particularly bare ice habitats, the studies conducted in this thesis regarding surface ice and glacier algae blooms were highly exploratory. The results produced, however, provide justification for further, more targeted research regarding the presence of active microbially mediated nutrient cycling, such as N fixation or the presence of phosphatase enzymes, in surface ice inhabited by glacier algae.

- **Limited investigation into microbial, particle interaction.** Results from Chapter 2 were limited by the lack of particle imagining post incubation due to small sample volume. Imaging, such as electron microscopy, of treated vs. untreated cryoconite particles would have been extremely useful in confirming the colonization of particles by the heterotrophic community. Using electron microscopy, investigations could have also been made into the potential physical processes of particle weathering utilized by the heterotrophs. Further investigations into microbial, particle interactions need to be made not only in snow pack environments but also ice surface environments, as a significant relationship between dust content and microbial abundance in surface ice has already been reported for the GrIS (Stibal et al., 2015).

- **Influence of UV radiation on degradation of organic matter and dissolved nutrients.** As UV radiation is extremely high in supraglacial environments and persists for 24-hours a day during the polar summer, further research should be conducted into its effect on dissolved nutrients and organic matter. Considering ammonium photoproduction from dissolved organic matter has already been found in several aquatic environments (Bushaw et al., 1996; Wang et al., 2000), and snow has been shown to act as a highly efficient photochemical reactor, particularly regarding the photolysis of nitrate (Grannas et al., 2007; Amoroso et al., 2010), it seems likely that the intense UV radiation in these environments during spring and summer may influence nutrient abundance and bioavailability. As the results from the PhD have shown surface ice inhabited by glacier algae to be areas of high organic nutrient and carbon accumulation, this further work seems highly vital to understanding nutrient dynamics in supraglacial environments.

- **Quantification of glacier algae C:N:P.** At present there is no reported C:N:P ratio for glacier algae. This remains a key gap in the literature as it would greatly enhance the current understanding of glacier algae's possible nutrient limitations and cycling potential, which could then be upscaled to influences on global biogeochemical cycles. Results in this thesis were limited by this lack of knowledge and thus relied on other C:N:P ratios, such as the Redfield ratio and other aquatic microbial cellular ratios, when assessing potential nutrient limitations to glacier algae blooms or the composition of extra polymeric substances.

5.4 Publications to date

Holland, A. T., Bergk Pinto, B., Layton, R., Williamson, C. J., Anesio, A. M., Vogel, T. M., Larose, C., and Tranter, M., (2020). Over winter microbial processes in a Svalbard snow pack: an experimental approach, *Frontiers in Microbiology*, 11, 1029, https://doi.org/10.3389/fmicb.2020.01029.

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5.5 Future publications

Holland, A. T., Williamson, C. J., Tedstone, A. J., Anesio, A. M., Tranter, M., (in prep). Dissolved N speciation and concentrations during snowmelt in the Dark Zone of the Greenland Ice Sheet: evidence for microbial activity in the snow pack and underlying ice during the spring thaw. *Biogeochemistry*.

Nicholes, M. J., Williamson, C. J., Tranter, M., **Holland, A. T.**, Yallop, M., Anesio, A. M., (in prep). Photodegradation and biodegradation of dissolved organic matter on the Greenland Ice Sheet. *Biogeosciences*.

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